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# Semen Quality in Men with Malignant Diseases before and after Therapy and the Role of Cryopreservation

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**Abstract**

***Objective:*** To review the literature and answer questions about semen quality in young cancer patients before and after therapy and the importance of sperm cryopreservation.
***Methods:*** All aspects of sperm cryopreservation and effects of therapies on semen quality were examined on the basis of MedLine database searches.
***Results:*** Chemotherapy, radiation, or their combination results in a significant reduction of sperm quality and as a consequence an indefinite time of infertility follows. The type of cancer and the pretreatment sperm concentrations were the most significant factors governing posttreatment semen quality and recovery of spermatogenesis. Due to their age, fertility and sexual functioning are key issues for these patients. Yet there is no medical protection of the germinal epithelium available. Male germ cell transplantation is in its infancy and still there are no therapeutical options to improve spermatogenesis after damage has occurred. Consequently, cryopreservation represents the only preemptive measure for conserving fertility.
***Conclusion:*** This manuscript updates the current knowledge of diverse chemotherapeutic treatment regimens and their gonadotoxic effects as well as the development of posttreatment fertility in cancer patients. The importance and rationale of sperm cryopreservation are discussed and possible future options are highlighted.


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## 1. Introduction

Approximately 15% of men with newly diagnosed cancer are younger than 55 yr at the time of diagnosis, and about 26% of them are <20 yr old [1]. Around 24,000 new cases of testicular cancer are diagnosed every year in Europe. It is the most common cancer in men younger than 45 yr, accounting for 17% of all cancers occurring in men below that age [2,3]. A recently published study documented continuing increases in incidence in 12 European countries [4].

Today, a high percentage of these patients are cured by using chemotherapy, radiation, or their combination. Side effects of antineoplastic agents and radiation are diverse and include a temporary or permanent influence on fertility. Due to their age, family planning has often not started yet. Hence, fertility and sexual functioning are key issues for these patients [5].

## 2. Methods

We conducted an extensive MedLine literature research (search terms "sperm cryopreservation," "pretreatment sperm quality," "posttreatment sperm quality," and "gonadotoxicity") for reports published from 1974 through 2007; only full-length English and French language articles identified during this search were considered for this analysis. The articles obtained were used for interpretation and critical analysis of results. Meta-analytic techniques were used within this review. Preference was given to articles with larger series and containing latest information. Attention was especially paid to therapies of cancer entities concerning young patients, such as testicular cancer and Hodgkin's disease.

## 3. Pretreatment sperm concentrations in cancer patients

Evidence suggests that the disease process itself influences spermatogenesis. Before starting any therapy, oligozoospermia is more often seen than in healthy men. Chung et al reported the presence of oligozoospermia in 28% of patients with testicular cancer, 25% with Hodgkin's lymphoma, 57% with leukemia, and 33% with gastrointestinal malignancies [6]. Other studies report semen abnormalities in 47–67% of patients with Hodgkin's disease. There was no correlation between semen abnormalities and disease stage or systemic symptoms [7].

The exact mechanism responsible for the decreased semen quality in cancer patients is not well established. Multiple factors are likely involved, including preexisting defects in germ cells and systemic effects of cancer [7].

Secretory substances of the tumor, such as hormones and cytokines, could influence spermatogenesis. The number of spermatogenesis defects was highest in the testicular tissue closest to the tumor [8]. Pretreatment impairment of spermatogenesis has been well studied in patients with Hodgkin's disease. It is hypothesized that observed systemic disturbances in the balance between subpopulations of T lymphocytes, which occur in patients with Hodgkin's disease, could be a cause of dyspermia [9].

Hallak et al reported poor sperm count and motility in patients with various cancers including central nervous system tumors. Poor quality in these patients may be a result of endocrine disturbances at central levels, systemic consequences of cancer, or both [10].

Defects in germ cells before therapy could also due to the decline of human fecundity as recently discussed [11].

## 4. Gonadotoxic effects of different cancer therapies

Drugs can freely reach Leydig and Sertoli cells and spermatogonia, which are at the outer rim of the tubules. Many chemotherapeutic drugs even penetrate the Sertoli cell barrier and damage late-stage germ cells; especially, differentiating spermatogonia proliferate most actively and are extremely susceptible to cytotoxic agents [12].

The basis for the recovery of spermatogenesis is formed by type A spermatogonia, which survived polychemotherapy if threshold cumulative cytostatic doses are not surpassed [13]. After cytotoxic therapies, germ cells frequently appear to be absent as a result of killing the spermatogenic stem cells, loss of the support of somatic cells, or a combination of both. Later-stage germ cells (spermatocytes onward) are relatively insensitive to killing. This is one reason the sperm count is not immediately affected by chemotherapy but often diminishes over time. During the first 2 mo of therapy, sperm counts may remain normal or be only moderately reduced. Three months after irradiation (time required for differentiating spermatogonia to become sperm) azoospermia often appears in patients given highly gonadotoxic agents. The induced azoospermia can be either temporary or prolonged, depending on the survival of stem spermatogonia and their ability to proliferate, differentiate, and produce spermatozoa. If the patient is treated with less toxic regimens, oligozoospermia or even normozoospermia may be maintained. As animal studies have shown, these

later-stage cells are susceptible to the induction of mutagenic damage and even can transmit mutations induced in their DNA to the next generation. Leydig and Sertoli cells do not proliferate in adults and survive most cytotoxic therapies. However, functional damage could occur [12,13].

If there is a loss of germ cells, inhibin secretion by Sertoli cells declines and because inhibin limits follicle-stimulating hormone (FSH) secretion by the pituitary, serum FSH rises. Because of interpatient variability in baseline levels, FSH measurements show only an imperfect correlation for sperm count. In contrast, a study by Petersen et al indicated more reliable of inhibin B levels [14].

Many factors influence male fertility after cancer treatment. Besides the drug itself, its mode of administration, disease, age, anatomic problems, primary or secondary hormonal insufficiency, size/location of the radiation field, dose, dose intensity, and pretreatment fertility of the patient play important roles [15].

### 4.1. *Chromosome abnormalities after chemotherapy*

Chromosome studies of testicular cancer patients before and up to 13 yr after BEP (bleomycin, etoposide, and cisplatin) chemotherapy demonstrated no significant difference in the frequency of numerical or structural chromosomal abnormalities comparing sperm karyotype analysis before and after therapy. Multicolor fluorescence in situ hybridization (FISH) analysis for chromosomes 1, 12, XX, and YY in sperm did not detect any significant differences in the frequencies of disomy but a significant increase in the frequency of 24, XY, and diploid sperm after chemotherapy [16].

The available evidence indicates that there is a significant risk of producing chromosomal abnormalities in sperm during and the first few weeks after chemotherapy in humans (similar to animal studies). It seems these defects decay with time [17,18].

Available data from offspring of cancer survivors are limited, representing diverse cancers, therapies, time to pregnancies, and reproductive outcomes. In a population-based study the adjusted proportion of live-born children in survivor families with abnormal karyotypes was the same as among the compared sibling families [19].

### 4.2. *Gonadotoxicity of antineoplastic agents*

Alkylating agents are associated with a high risk of azoospermia [20]. As one of them, cisplatin is at present the main chemotherapeutic agent in oncologic protocols for testicular tumors. Damage to both spermatogenesis and testicular endocrine function will be temporary, provided that the cumulative cisplatin dose does not exceed 400 mg/m$^2$ (or 600 mg/m$^2$, according to other authors). Combination with ifosfamide (42 g/m$^2$) seems to increase toxicity [12,20].

Popular treatment regimens for patients with Hodgkin's disease are MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) and ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine). Particularly procarbazine (4 g/m$^2$) is associated with prolonged azoospermia. Doxorubicin (770 mg/m$^2$), vincristine (8 g/m$^2$), and vinblastine (50 g/m$^2$) can be additive if combined with strong toxic agents, but cause only temporary reductions in sperm counts if given alone [12].

Table 1 presents an update of diverse agents and their effect on spermatogenesis. There are unknown effects on sperm production for a number of new agents, such as taxanes or monoclonal antibodies [21].

### 4.3. *Gonadotoxicity of irradiation*

Multiple variables influence gonadotoxicity of irradiation. In addition to the radioactive source, variables such as direct or diffused irradiation, total applied dose, number and duration of fraction, individual variables, such as individual susceptibility and age of the patient, are factors also.

During abdominopelvic radiotherapy the diffused testicular dose is estimated to be 1–2% of the total dose applied to the tumor. Scrotal irradiation without testicular protection is constantly associated with azoospermia [20].

Doses $>$0.15 Gy are required to produce any reduction in sperm count. Direct irradiation (0.15–0.35 Gy) causes oligozoospermia; doses between 0.35 Gy and 0.5 Gy cause reversible azoospermia. The nadir of sperm count occurs 4–6 mo after the end of treatment and 10–18 mo are required for complete recovery. However, doses of $\geq$1.2 Gy are associated with a reduced risk of recovery of spermatogenesis. Time to recovery is also likely to depend on the dose. Cumulative doses of fractionated radiotherapy of $>$2.5 Gy generally result in prolonged and likely permanent azoospermia. Applied doses $>$6 Gy cannot be tolerated by spermatozoa. Leydig cells are damaged by direct testicular irradiation of $>$15 Gy. The damage is irreversible if doses are $>$20 Gy [12,20,22].

Differently from other organ systems, where fractionation of radiation reduces damage, radiation doses to the germinal epithelium of the testis given in 3- to 7-wk fractionated courses cause more

**Table 1 – Effects of antineoplastic agents on sperm production**

| Type of agent | Chemotherapy agent | Effect on fertility | Mechanism of action |
|---|---|---|---|
| Alkylating agents | Cyclophosphamide | Prolonged azoospermia [12] | A |
| | Chlormethine | Prolonged azoospermia [12] | A |
| | Chlorambucil | Prolonged azoospermia [12] | A |
| | Mephalan | Prolonged azoospermia [12] | A |
| | Ifosfamide | Azoospermia likely, but always given with other highly sterilizing agents [12] | A |
| | Busulfan | Azoospermia likely, but always given with other highly sterilizing agents [12] | A |
| | Carmustine | Azoospermia in adulthood after treatment before puberty [12] | A |
| | Thiotepa | Could cause prolonged azoospermia if added to other agents. Only temporary reductions in sperm count when not combined [12] | A |
| | Dacarbazine | Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | B |
| Anti-metabolites | Mercaptopurine | Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | B |
| | Azathioprine | No changes in semen parameters were noted [73] | B |
| | Fludarabine | Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | C |
| | Methotrexate | Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | D |
| | 5-Fluorouracil | Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | E |
| | Gemcitabine | In male mice moderate to severe reduced fertility [74] | E |
| | Cytarabine (Ara-C®) | The potential effects on sperm counts, function, and fertility have not been studied yet | F |
| Methylhydrazines | Procarbazine | Prolonged azoospermia [12] | G |
| Cytotoxic Antibiotics and related substances | Dactinomycin | Azoospermia likely, but always given with other highly sterilizing agents [12] | H |
| | Bleomycin | Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | I |
| | Daunorubicin | Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | J |
| | Epirubicin | Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | J |
| | Mitoxantrone | Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | J |
| | Doxorubicin (Adriamycin®) | Could cause prolonged azoospermia if added to other agents. Only temporary reductions in sperm count when not combined [12] | J |
| | Mitomycin | Intraperitoneal administration to male mice decreased sperm production, sperm count and sperm motility [74] | K |
| Platinum compounds | Cisplatin | Prolonged azoospermia [12]. Damage can be irreversible [20]. | L |
| | Carboplatin | Azoospermia likely, but always given with other highly sterilizing agents [12] | L |
| | Oxaliplatin | Prolonged azoospermia not often observed at indicated dose [12]. Higher probability of recovery [23]. | L |

**Table 1 (*Continued*)**

| Type of agent | Chemotherapy agent | Effect on fertility | Mechanism of action |
|---|---|---|---|
| Monoclonal antibodies | Alemtuzumab | The potential effects on sperm counts, function, and fertility have not been studied yet | [M] |
| | Rituximab | The potential effects on sperm counts, function, and fertility have not been studied yet | [N] |
| | Gemtuzumab ozogamicin | The potential effects on sperm counts, function, and fertility have not been studied yet | [O] |
| | Bevacizumab | The potential effects on sperm counts, function, and fertility have not been studied yet | [P] |
| Plant Alkaloids and other natural products | Vinblastine | Could cause prolonged azoospermia if added to other agents. Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | [Q] |
| | Vincristine | Could cause prolonged azoospermia if added to other agents. Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | [Q] |
| | Etoposide | Only temporary reductions in sperm count at doses used in conventional regimens, but additive effects are possible [12] | [R] |
| | Paclitaxel | In male mice significantly reduced fertility. Testicular degeneration observed in rodents [74]. | [S] |
| | Docetaxel | No impairment of fertility in rats (1/50 of human dose) but testicular degeneration [74] | [S] |
| Protein kinase inhibitors | Sorafenib | The potential effects on sperm counts, function, and fertility have not been studied yet | [T] |
| | Imatinib mesylate | In male rats testicular weights and percent of motile sperm were decreased [12] | [U] |
| | Dasatinib | The potential effects on sperm counts, function, and fertility have not been studied yet | [V] |
| Enzymes | L-Asparaginase | The potential effects on sperm counts, function, and fertility have not been studied yet | [W] |

[A] Attachment of alkyl groups to DNA bases, resulting in the DNA being fragmented by repair enzymes in their attempts to replace the alkylated bases, preventing DNA synthesis and RNA transcription from the affected DNA. DNA damage via the formation of cross-links which prevents DNA from being separated for synthesis or transcription Induction of mispairing of the nucleotides leading to mutations.
[B] Antagonizes purine metabolism and may inhibit synthesis of DNA, RNA, and proteins.
[C] Metabolite appears to act by inhibiting DNA synthesis.
[D] Inhibition of folic acid reductase, leading to inhibition of DNA synthesis and inhibition of cellular replication.
[E] Pyrimidine analog.
[F] Inhibition of DNA polymerase. Direct DNA damage and incorporation into DNA (primarily killing cells undergoing DNA synthesis).
[G] May directly damage DNA. Hydrogen peroxide (formed during the auto-oxidation of the drug) may attack protein sulfhydryl groups contained in residual protein which is tightly bound to DNA.
[H] Bind strongly, but reversibly, to DNA, interfering with synthesis of RNA and with protein synthesis.
[I] Single-strand and double-strand breaks in DNA.
[J] Affects dividing and nondividing cells via inhibition of DNA synthesis and repair. DNA intercalation. DNA topoisomerase II inhibition.
[K] Bifunctional and trifunctional alkylating agent. Binding to DNA leads to cross-linking and inhibition of DNA synthesis and function.
[L] Attachment of alkyl groups to DNA bases, resulting in the DNA being fragmented by repair enzymes in their attempts to replace the alkylated bases, preventing DNA synthesis and RNA transcription from the affected DNA. DNA damage via the formation of cross-links which prevents DNA from being separated for synthesis or transcription. Induction of mispairing of the nucleotides leading to mutations.
[M] Binds to CD52, a nonmodulating antigen.
[N] Binds to the CD20 antigen. Antibody appears to induce apoptosis.
[O] Directed against the CD33. After binding and internalization, derivative binds to DNA, resulting in DNA double strand breaks and cell death.
[P] Binds VEGF and prevents interaction of VEGF.
[Q] Inhibition of mitosis at metaphase through its interaction with microtubular proteins of the mitotic spindle, leading to crystallization of the microtubule and mitotic arrest or cell death.
[R] Inhibits DNA topoisomerase II, thereby inhibiting DNA synthesis at the premitotic stage of cell division.
[S] Interfere with the normal function of microtubule growth. This destroys the cell's ability to use its cytoskeleton in a flexible manner.
[T] Interacts with multiple intracellular and cell surface kinases.
[U] Protein-tyrosine kinase inhibitor. Inhibits proliferation and induces apoptosis in Bcr-Abl positive cell lines.
[V] Tyrosine Kinase Inhibitor.
[W] Destroys asparagine external to the cell.

**Table 2 – Effects of diverse cancer treatments on male fertility**

| Tumor entity | Treatment | Posttreatment Fertility | Reference |
|---|---|---|---|
| Testicular cancer | Cisplatin/carboplatin | Normozoospermia in 64% at 1 yr in 80% at 3–5 yr, there was a higher probability of recovery in carboplatin-based therapy | [23] |
| | | Normozoospermia in 50% at 2 yr Based therapy and 80% at 5 yr | [22] |
| | Cisplatin 400 mg/m$^2$ plus ifosfamide 30 g/m$^2$ | Prolonged azoospermia in 55% | [12] |
| | Para-aortic irradiation | No azoospermia | [24] |
| | | Elevated FSH/LH values if irradiated by hockey stick fields (scatter dose <0.55 Gy) | [25] |
| | Single-dose irradiation <0.5 Gy | Reversible oligozoospermia | [12] |
| | Single-dose irradiation (<1 Gy) | Oligozoospermia (recovery <18 mo) | [72] |
| | Single-dose irradiation (<3 Gy) | Azoospermia (recovery <30 mo) | [72] |
| | Single-dose irradiation (<5 Gy) | Azoospermia (recovery >5 yr) | [72] |
| | Fractionating irradiation >0.1 Gy | Reversible oligozoospermia | [12] |
| | Fractionating irradiation >0.3 Gy | Temporary azoospermia | [12] |
| | Fractionating irradiation >2.5 Gy | Prolonged azoospermia | [12] |
| | Fractionating irradiation 16–18 Gy | SCO syndrome in 95% | [26] |
| Hodgkin's disease | MVPP | Azoospermia in >90% | [22] |
| | MOPP (≥6 courses) | Azoospermia in >90% | [22] |
| | | Prolonged azoospermia in 85% | [12] |
| | | 100% azoospermia <14 mo, 10% normal spermiogram after decades | [29] |
| | | 89% azoospermia after 1 yr | [31] |
| | MOPP (≤3 courses) | Recovery significant higher compared to ≥5 courses | [30] |
| | ChlVPP/EVA hybrid | Azoospermia in >90% | [22] |
| | COPP (4–9 courses) | Azoospermia in >90% | [22] |
| | ABVD | Temporary azoospermia with normal sperm count in all at 18 mo | [22] |
| | | 90% normozoospermia after 1 yr | [31] |
| | VEBEP plus INF radiotherapy | Reversible damage in 50% | [32] |
| Non-Hodgkin's lymphoma | CHOP | Permanent azoospermia in ~30% | [22] |
| | VAPEC-B | Normozoospermia in >95% | [22] |
| | VACOP-B | Normozoospermia in >95% | [22] |
| | MACOP-B | Normozoospermia in >95% | [22] |
| | VEEP | Normozoospermia in >95% | [22] |
| Bone marrow transplant for a variety of malignancies | CY (19 g/m$^2$) alone | Recovery of spermatogenesis in 90% | [33] |
| | CY plus BU or TH | Recovery of spermatogenesis in 50%, in the majority the sperm quality was severely impaired | [33] |
| | CY plus TAI/TBI | Recovery of spermatogenesis in 17%, never before the 4th year after transplantation | [33] |
| | CBV | FSH raised in >95% | [22] |
| | High-dose melphalan (140 mg/m$^2$) | FSH raised in >95% | [22] |
| | | Prolonged azoospermia | [12] |
| | BEAM | 100% azoospermia (follow-up 1–111 mo after transplant) | [34] |
| Superficial bladder cancer | Intravesical instillation of MMC | Insignificant changes in sperm quality | [38] |
| | Intravesical instillation of BCG | Oligozoospermia | [38] |

**Table 2 (*Continued*)**

| Tumor entity | Treatment | Posttreatment Fertility | Reference |
|---|---|---|---|
| High-risk superficial bladder cancer | Nerve- and seminal-sparing radical cystectomy | 87.5% semen retrieval via urine | [39] |
| Prostate cancer | Brachytherapy (exposure of 10 mR/h at the symphysis pubis) | No change in semen parameters | [36] |
| | External beam radiotherapy (70 Gy to prostate bed) | Damage on spermatogenesis | [37] |
| Osteosarcoma | Ifosfamide 46 g/m$^2$ plus cisplatin 560 g/m$^2$ plus doxorubicin plus methotrexate | Patients who received high-dose ifosfamide showed a higher incidence of azoospermia | [41] |
| Ewing sarcoma or soft tissue sarcoma | CYADIC/CYVADIC | 40% of men recovered to normospermic levels by 5 yr after treatment | [75] |
| Thyroid cancer | Treating with high dose of radioiodine $^{131}$I | No evidence of infertility | [42] |
| Rectal cancer | Pelvic radiotherapy (50 Gy) | High risk of permanent infertility and risk of endocrine failure (hypogonadism) | [43] |

ABVD = doxorubicin hydrochloride, bleomycin, vinblastine, and dacarbazine; BCG = bacille Calmette-Guérin; BEAM = carmustine, etoposide, Ara-C, and melphalan; BU = busulphan; CBV = cyclophosphamide, carmustine, and etoposide; ChlVPP/EVA = chlorambucil, vinblastine, prednisolone, procarbazine, doxorubicin, vincristine, and etoposide; CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisolone; COPP = cyclophosphamide, vincristine, procarbazine, and prednisolone; CY = cyclophosphamide; CYADIC = cyclophosphamide, doxorubicin, and dacarbazine; CYVADIC = vincristine added to CYADIC; FSH = follicle-stimulating hormone; Gy = Gray; INF = involved nodal field; LH = luteinizing hormone; MACOP-B = mustine in place of vinblastine; MMC = mitomycin C; MOPP = mustine, vincristine, procarbazine, and prednisolone; MVPP = mustine, vinblastine, procarbazine, and prednisolone; SCO = Sertoli cell only; TAI = thoracoabdominal irradiation; TBI = total body irradiation; TH = thiotepa; VACOP-B = vinblastine, doxorubicin, prednisolone, vincristine, cyclophosphamide, and bleomycin; VAPEC-B = vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide, and bleomycin; VEBEP = etoposide, epirubicin, bleomycin, cyclophosphamide, and prednisolone; VEEP = vincristine, etoposide, epirubicin, and prednisolone.

gonadal damage than single doses. An irreversible damage of the spermatogenesis will begin at a cumulative dose of >2.5 Gy when the radiation is fractionated [12].

As yet, no significant studies have assessed the impact of innovative forms of radiotherapy (eg, tomotherapy), on either germ cell preservation or sperm retrieval. Even though results remain to be seen, these new treatment modalities are supposed to lower toxicity.

## 5. Recovery of fertility after treatment

The type of cancer or disease and pretreatment sperm concentrations are the most significant factors governing posttreatment sperm parameters and recovery of spermatogenesis. Unfortunately, many studies evaluating fertility after cancer therapy lack of pretreatment assessments of semen parameters as well as data regarding the hormonal milieu in male patients. Table 2 gives an overview of posttreatment fertility after diverse cancer therapies.

### 5.1. *Recovery in patients with testicular cancer*

One year after orchiectomy and cisplatin-based chemotherapy normozoospermia was seen in 64% of all patients and after 3–5 yr, recovery was found in 80%. Even after 5 yr, normalization could be seen. These results depended, however, on the pretreatment status of fertility and the patient's age. Some patients had irreversible infertility. If patients were treated with carboplatin-based rather than cisplatin-based therapy, the probability of recovery was significantly higher [23]. No azoospermia was seen after orchiectomy and para-aortic irradiation in type IA and IIA seminoma and high recovery could be observed [24]. Comparing patients with stage I seminoma irradiated by hockey stick fields (scatter dose 0.55 Gy) or limited radiotherapy (scatter dose 0.25 Gy) 4 yr after their treatment, the first group showed significantly elevated FSH and luteinizing

hormone (LH) values but no changes in sperm parameters [25]. After exploring 40 patients undergoing testicular radiation (16–18 Gy) because of testicular intraepithelial neoplasia (TIN) lesions, spermatogonia could be found in only 2 of them. The others showed a Sertoli cell-only (SCO) syndrome confirmed through testicular biopsy [26].

There was a statistically significant decrease in sperm parameters in testicular cancer patients, who cryobanked about 1 mo after the removal of the cancerous testis and before beginning therapy [27].

Analysis showed that patients with testicular carcinoma had the lowest pretreatment sperm concentrations but also the lowest incidence of azoospermia after treatment compared to other cancer entities [28].

### 5.2. *Recovery in patients with Hodgkin's disease*

Examining fertility in 47 men with Hodgkin's disease after MOPP chemotherapy, Marmor et al reported of azoospermia in all patients 14 mo after the end of therapy. In some patients regeneration started after 5 yr, in some even after 10 yr. Only one of 11 patients with a long-term follow-up demonstrated a nearly normal semen analysis [29]. The results of other studies suggest that three cycles of MOPP chemotherapy represent a maximum exposure compatible with the recovery of spermatogenesis [30].

The use of the ABVD regimen seems to induce fewer detrimental effects on sperm production compared to MOPP therapy. A comparative study done 1 yr after ending chemotherapy showed that 90% of patients treated with ABVD had no change in their sperm count. Compared to those treated with MOPP, eight of nine were azoospermic [31].

Gonadal damage was evident in a large majority of male patients who underwent cycles of VEBEP (etoposide, epirubicin, bleomycin, cyclophosphamide, and prednisolone) followed by radiotherapy (30–36 Gy) to the nodal site or sites of pretreatment disease. In 50% of the patients this damage was reversible [32].

### 5.3. *Recovery in patients with non-Hodgkin's lymphoma*

Chemotherapy regimens used for treatment of non-Hodgkin's lymphoma (NHL) are generally less gonadotoxic than those used for Hodgkin's disease, probably related to the absence of procarbazine in the standard regimens. All patients treated with CHOP-based (cyclophosphamide, doxorubicin, vincristine, and prednisolone) chemotherapy were azoospermic during treatment, but 67% recovered after 5 yr [22].

### 5.4. *Recovery after bone marrow transplantation*

In 90% of patients conditioned with cyclophosphamide alone, recovery of spermatogenesis could be observed. This rate declined to 50% if busulphan or thiotepa was added and to 17% if patients underwent total body irradiation (TBI) or thoracoabdominal irradiation (TAI). Following cyclophosphamide combined with TBI/TAI, recovery of spermatogenesis never occurred before the fourth year after transplantation and was observed up to 9 yr. The overall incidence of azoospermia was 70.3% [33].

All patients conditioned with a combination of carmustine, etoposide, cytosine arabinoside, and melphalan (BEAM) showed azoospermia on follow-up 1–111 mo after transplantation [34].

### 5.5. *Recovery after different treatments of prostate cancer*

In a recent questionnaire-based study, only 3.7% of patients listed fertility as the major concern due to their age [35]. But for several reasons, including earlier diagnosis and screening programs, the prevalence of prostate cancer is growing in young patients. To avoid testicular sperm extraction (TESE) after radical prostatectomy, sperm cryopreservation is mandatory in young patients. In case reports of patients who underwent brachytherapy (exposure of 10 mR/h at the symphysis pubis) no significant changes in sperm parameters occurred [36]. This is contrary to several reports of external-beam radiation therapy, which is associated with decreased spermatogenesis [37].

### 5.6. *Recovery after different treatments of bladder cancer*

The treatment of 12 young patients with superficial transitional cell carcinoma by adjuvant intravesical application of either bacille Calmette-Guérin (BCG) or mitomycin C (MMC) showed normal volume ejaculate in all except one patient. Six who received MMC had only a few minor insignificant changes in sperm quality, different from BCG where three of six showed remarkable changes in all sperm quality parameters [38].

In patients with high-risk superficial bladder cancer who underwent nerve- and seminal-sparing radical cystectomy, fertility potential with sperm retrieval via urine was reported in seven of eight patients [39].

### 5.7. *Recovery after treating pediatric sarcomas*

The reported infertility rate in young men treated for pediatric sarcomas with multimodality therapy was

76% [40]. Longhi et al showed a correlation between the ifosfamide dosage and fertility recovery in male patients with osteosarcoma. There were no congenital malformations seen in the children of the few patients who fathered children [41].

### 5.8. *Recovery after radioiodine therapy*

There is an excellent long-term prognosis if young men with differentiated thyroid cancer are treated with high doses of radioiodine $^{131}$I. In a prospective study of 14 consecutive patients receiving radiation for thyroid cancer, the gonadal function was assessed by serum FSH, LH, and testosterone measurements. Radiation dose to the testes was measured by thermoluminescent dosimetry. Altogether radioiodine treatment for thyroid cancer may result in transient impairment of gonadal function. However, no evidence of infertility was found, even if patients required multiple administrations for persistent or metastatic thyroid cancer [42].

### 5.9. *Recovery after radiotherapy of rectal cancer*

In 11 patients (mean age, 55.2 yr) the dose received by the testicles during pelvic radiotherapy for rectal cancer (50 Gy) was measured. The mean cumulative radiation exposure to the testicles was 3.56 Gy (0.7–8.4 Gy), thus it is assumed that a high risk of permanent infertility and a risk of hypogonadism will occur [43].

## 6. Sperm cryopreservation

### 6.1. *Incidence of sperm cryopreservation and its value for cancer patients*

Depending on different surveys, 50–70% of cancer patients wanted children in the future, including 77% who were childless at the time cancer was diagnosed [44,45]. Despite some anxieties about their own survival and risks to their children's health, men felt that the experience of cancer increased the value they placed on family closeness and would make them better parents [46].

Cryopreservation represents the only preemptive accompanying possibility for conserving fertility in young cancer patients [47]. However, only 24% of young cancer patients banked sperm, including 37% of childless men. The most common reason for failing to bank sperm was a lack of information [44]. Edge et al reported that only 67% of young men awaiting cancer therapy were enlightened about its possibility [48] and only 51% had been offered sperm banking [44].

In a questionnaire, 91% of 718 consulted oncologists agreed that sperm banking should be offered to all men before treatment, but 48% either never bring up the topic or mention it to <25% of eligible men. Neither the knowledge about cryopreservation nor seeing large numbers of eligible men increased the likelihood of discussing this option. The arguments cited were lack of time for the discussion, high costs, and lack of convenient facilities [49].

When pretreatment sperm cryopreservation was offered to young and middle-aged patients with newly diagnosed testicular cancer, about 50% of them were interested [50]. A positive psychological effect was reported of 80% of interviewed patients who banked sperm. Especially if sperm was banked on the patient's own initiative, it was found to offer encouragement during therapy [45].

### 6.2. *Effects of cryopreservation on semen quality*

Sixty percent of the patients worried about infertility despite having their sperm cryopreserved [45]. Freezing can have detrimental effects on a variety of sperm functions, some of them not accessible to the conventional semen analysis. Overall sperm quality deteriorated after cryopreservation. The damage by cryopreservation of spermatozoa will not be per se, but already existing defects present in the sperm subpopulation could be enhanced [51]. But duration seems not to be the main problem because probes can be stored in liquid nitrogen over decades without loss of quality. It is mainly the process of freezing in which the spermatozoa are damaged. Both sperm concentration and motility suffer over the time with the temperature kept at −196 °C [52].

In the future, there might be the possibility of less expansive dry storage of sperm as preliminary results are suggesting [53].

## 7. Surgical retrieval of spermatozoa in cancer patients

### 7.1. *TESE*

If azoospermia occurs in cancer patients before starting therapy, TESE could be a useful technique to obtain spermatozoa and must be discussed. As many studies showed, there are no predictable parameters for sperm retrieval in patients with nonobstructive azoospermia [54]. In 50% of azoospermic men with testicular cancer or malignant lymphomas, spermatozoa could be extracted by

TESE [55]. If any spermatozoa could be found, even round spermatids could be used for assisted reproductive technology (ART), because case reports have shown successful fertilizations [56].

### 7.2. *Using orchiectomized testis for sperm retrieval*

Inguinal orchiectomy remains the standard management for testicular mass. If the patient was azoospermic, successful intraoperative sperm retrieval by testicular microdissection of the orchiectomized testis could be performed simultaneously [57]. Alternatively sperm retrieval by aspirating sperm from the vas deferens and epididymis after orchiectomy was reported to be safe—tumor cell spillage could be avoided if done ex vivo—and successful [58].

## 8. Success rate of ART

Intracytoplasmic sperm injection (ICSI) has revolutionized the treatment of male infertility because minimal requirements for semen quantity either in the ejaculate or testicular tissue can be abandoned. Results indicate that surgically retrieved spermatozoa can be efficiently used for ICSI after freezing and thawing without compromising the outcome compared to fresh spermatozoa [59,60].

The fertilization rate in patients undergoing ICSI was slightly higher comparing fresh to cryopreserved sperm, but implantation and pregnancy rates were similar. Hypothetically, semen with abnormalities in motility might be more susceptible to cryopreservation damage, resulting in a lower fertility rate. Once the oocyte is fertilized, there seem to be no further negative influence on the outcome [61].

A follow-up survey of posttreatment paternity of 1433 men, all long-term survivors of testicular cancer, documented 554 men attempting conception. Among these men the overall 15-yr actuarial posttreatment paternity rate was 71% without the use of cryopreserved sperm and 48% if high-dose chemotherapy was used. ARTs were used by 22% of the couples who attempted conception after treatment [62].

Altogether 58% of cancer survivors with unwillingly childlessness due to antineoplastic treatment and who decided on ARTs used cryopreserved sperm. The delivery rate per cycle was similar using fresh or cryopreserved spermatozoa [63].

According to Saito et al no patients wanted to use cryopreserved sperm for fathering children if their spermatogenesis was restored, whether the majority recommended sperm cryopreservation or not [45].

## 9. Future therapeutic options

### 9.1. *Male germ cell and testicular stem cell transplantation*

As recently shown, male germ cell transplantation can be successfully done in animals [64,65]. Possibly in the future fertility restoration in young cancer patients could be done by harvesting germ cells before gonadotoxic therapy, cryopreserve them, and retransplant them after therapy [66,67]. Whether testicular germ cell transplantation will be put into practice depends on several restrictions across Europe due to a number of different legal limitations.

### 9.2. *Banking of testicular tissue from prepubertal boys*

A recent published study showed that prepubertal testicular tissue from boys facing gonadotoxic treatment could be cryobanked under special conditions. This could be the crucial step in fertility preservation for young patients in the future [68].

### 9.3. *Trials on medical protection of the germinal epithelium*

Protective effects of luteinizing hormone-releasing hormone analogs (LHRHAs) during gonadotoxic therapies have been examined in men. In contrast to animal studies, LHRHAs could not significantly influence severity and duration of germ cell impairment [69,70].

## 10. Conclusions

Chemotherapy, radiation, or their combination results in a significant reduction of sperm quality and as a consequence an indefinite time of infertility follows [12]. The tumor entity and pretreatment sperm concentrations were the most significant factors governing posttreatment semen quality and recovery of spermatogenesis. Keeping in mind that testicular stem cell transplantation may be a potential treatment for infertility, sperm cryopreservation still represents the only preemptive accompanying possibility for conserving fertility in cancer patients [47]. Because of a lack of information, only 48–67% of young men awaiting cancer therapy were enlightened about its possibility [48] and only 24% of young cancer patient cryobanked sperm [44]. Even if the cryopreserved sperm will not be used, as in most cases, a positive psychological effect will be achieved [45]. Cryopreservation should be considered as early as possible during treatment

planning, even in cases where the individual's risk of posttreatment infertility might seem to be minimal. If azoospermia is present before cancer therapy, in 50% TESE could be successful [55]. Analyses showed that sperm preservation is possible over decades [52] and similarity shown in pregnancy rates clearly indicate that cryopreserved sperm is not inferior to fresh sperm [60]. If no sperm were cryopreserved before treatment and the patient suffers from aspermia/azoospermia after treatment, TESE is the only possibility to harvest spermatozoa. At present there is no established method to stimulate impaired spermatogenesis after treatment. Sperm retrieval is positive in 92% of men with aspermia and 58% of men with nonobstructive azoospermia [71].

## Conflicts of interest

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