Use of cryopreserved ovarian tissue in the Danish fertility preservation cohort

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Objective: To evaluate the use of cryopreserved ovarian tissue in the Danish fertility preservation cohort.
Design: Retrospective cohort study.
Setting: University hospitals and fertility clinics.
Patient(s): Ovarian tissue cryopreservation (OTC) was performed for 1,186 Danish girls and women from 1999–2020, of whom 117 subsequently underwent ovarian tissue transplantation (OTT). Subgroup 1 included 759 patients with a follow-up period of >5 years. Out of these, OTT rates were further analyzed for those patients who were alive and aged >24 years in July 2020 (subgroup 2; n = 554).
Intervention(s): OTC and OTT.
Main Outcome Measure(s): OTT, death, donation of tissue.
Result(s): In subgroup 1, 14% of the patients had undergone OTT, 18% had died, 9% had donated their tissue for research, and 59% still had their tissue stored. In subgroup 2, 19% had undergone OTT and for most diagnoses the OTT rates ranged from 15% to 22% with benign hematologic diseases having the highest OTT rate (35%). On the basis of the entire cohort, stratified age analysis indicated that women aged ≥30 years at OTC were more likely to return for OTT than women aged 18–29 years at OTC; mean storage times were 3.7 and 3.6 years, respectively. Only 4% of the girls aged <18 years at OTC had undergone OTT.
Conclusion(s): The OTT rates depended on the diagnosis, age at OTC, and follow-up time. Specific criteria are needed for reporting and comparing OTT rates. Six out of 10 patients still had their cryopreserved tissue stored and longer follow-up is needed, especially for younger girls. (Fertil Steril® 2021;116:1098-106. ©2021 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Fertility preservation, ovarian tissue cryopreservation, ovarian tissue transplantation, return rate, follow-up

With >130 children born worldwide and live birth rates of approximately 20%–30% per woman with transplanted ovarian tissue, ovarian tissue cryopreservation (OTC) has become a valid method to preserve fertility in young girls and women (1–7). Thousands of young women have had their ovarian tissue cryopreserved, and >360 ovarian tissue transplantations (OTTs) were reported in 2018 (8). However, on the basis of current knowledge, the return rate appears to be low, as only approximately 5% of the patients have undergone OTT as reported by cohort studies (4, 7, 9–12).

The low rate of OTT reflects that some women remain fertile after gonadotoxic treatment (13), some do not survive their cancer (14), whereas

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others have no desire to reproduce because of their young age at OTC or medical or social reasons. Previous studies showed that premature ovarian insufficiency (POI; i.e., treatment-induced menopause) appears to occur in 22%–33% of all patients after OTC (4, 9, 11, 13). Thus, spontaneous recovery of ovarian function and fertility after oncologic treatment is not unusual, but the long-term risk of POI is still not clarified. Furthermore, OTT is contraindicated in some patients because of a presumed risk of reintroducing the malignancy or because the women may have difficulties carrying a pregnancy. The low usage rate of cryopreserved ovarian tissue resembles that of cryopreserved oocytes and sperm samples stored for fertility preservation (12, 15, 16).

It is currently not possible to accurately predict which patients will benefit the most from OTC and OTT. Nonetheless, studies have reported a very high patient satisfaction rate even for those patients who did not require OTT (4, 11, 13). Moreover, in a recently published qualitative study, OTC was connected with positive experiences as it created future-oriented believe and reproductive possibilities for the patients (17). Thus, the importance of fertility preservation for young women in a situation of severe crisis and facing potentially sterilizing treatment should not be underestimated.

Large cohort studies are needed to reveal the "true" return rates for young girls and women undergoing OTC and to identify factors predicting the subsequent use of cryopreserved ovarian tissue. The Danish fertility preservation program for OTC was initiated in 1999, and some women with cryopreserved ovarian tissue are now approaching the age of natural menopause. This allows for a 20-year descriptive analysis of the fate of cryopreserved ovarian tissue in the Danish cohort, which is one of the largest and oldest OTC cohorts to date. On the basis of almost 1,200 OTCs and 117 OTTs, the aim of the present study was to evaluate the use of cryopreserved ovarian tissue in relation to the diagnosis and the age of the patient. Usage was analyzed in terms of cryopreserved ovarian tissue being transplanted, discarded because of the death of the patient, donated for research purposes, or still in storage. This information is important to identify those patients who may or may not benefit from the procedure.

MATERIALS AND METHODS

Study Population

The study population included all 1,186 girls and women undergoing OTC for fertility preservation from 1999 to 2020 at the Laboratory of Reproductive Biology in Copenhagen, Denmark. All patients consented to OTC and underwent unilateral oophorectomy at one of the three referring hospitals in Denmark (Aarhus University Hospital, Copenhagen University Hospital, and Odense University Hospital). Ovarian tissue cryopreservation for fertility preservation was approved by the Ministry of Health in Denmark (J. no. J/KF/01/170/99) and is considered a standard treatment. The slow-freezing procedure was performed in all cases with a consistently high follicular survival (18, 19). Data collection regarding the death of patients was approved by the Danish Patient Security Authority (3-3013-2790/1).

Analysis

Information regarding the status of the stored tissue (i.e., transplanted, discarded, donated, or still in storage) and the current age of the patients were obtained on July 1, 2020. The data were stratified according to the diagnosis and patient age at the time of OTC. Mean values are reported with standard deviations (± SD). Comparisons of the time between the OTC and the final event (transplantation, patient's death, or donation of the tissue) were illustrated using Kaplan-Meier curves made in the statistical program R (version 3.4) on the basis of the data from the entire cohort. Probability plots for OTT were on the basis of the data of women who were still alive in July 2020 and depicted in relation to the storage time, in years, after OTC or age at OTT.

Separate analyses were done for a large subgroup of women with >5 years of follow-up (subgroup 1). This subgroup included patients undergoing OTC from 1999 to 2015 (n = 759). Of the 759 women in subgroup 1, return rates for OTT were further analyzed in a second subgroup (subgroup 2), which included patients who were alive and aged > 24 years in July 2020 (n = 554).

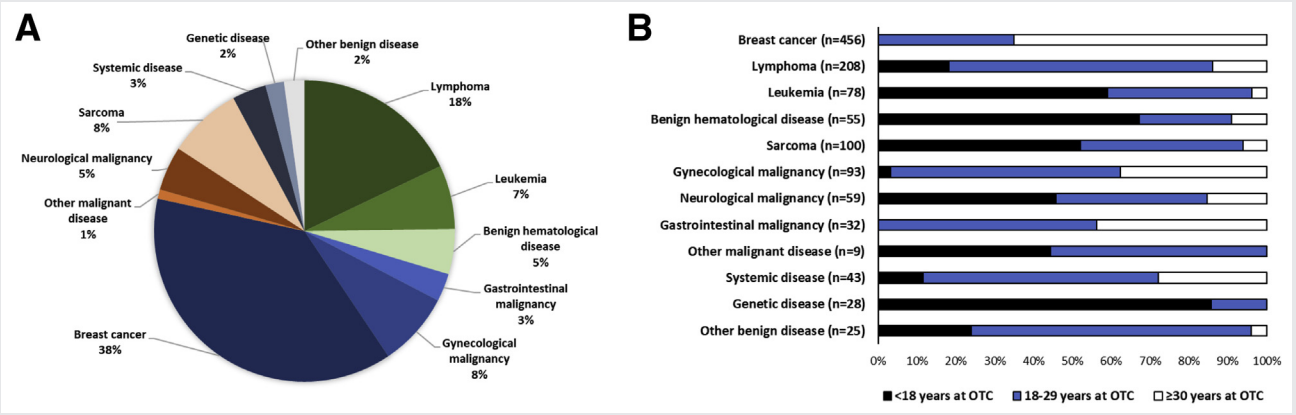
RESULTS

Diagnoses and Age at OTC in the Danish Cohort

The mean age of the patients undergoing OTC was 25.1 ± 9 years, ranging from 4 months to 43 years. Of the 1,186 patients, 21% (n = 242) were ≤18 years of age at the time of OTC, 70% (n = 833) were aged 19–34 years at OTC, and 9% (n = 111) were ≥35 years. The most prevalent diagnoses were breast cancer (38%) and hematologic diseases (30%) including lymphoma (18%), leukemia (7%), and benign hematologic diseases like thalassemia and sickle cell disease (5%) (Fig. 1A). In addition, malignant indications included sarcoma (8%), gynecologic malignancies (8%), neurologic malignancies (5%), gastrointestinal malignancies (3%), and other malignant diseases including kidney and nasopharyngeal cancer (1%). Benign conditions accounted for 12% of all the indications (including benign hematologic diseases [5%]), with systemic diseases including rheumatologic and autoimmune diseases in 3%, genetic diseases including Turner syndrome and galactosemia in 2%, and other benign diseases including ovarian conditions and a family history of POI in 2% of the patients (Fig. 1A). Figure 1B shows the distribution of the diagnoses in relation to the age groups, i.e., <18 years, 18–29 years, and ≥30 years. More than 97% of the patients with breast cancer and gynecological and gastrointestinal malignancies were >18 years of age at OTC, whereas most patients (46%–86%) with genetic diseases, benign hematologic diseases, leukemia, sarcomas, and neurologic malignancies were <18 years of age at OTC.

Supplemental Table 1 (available online) shows that during the 20-year period, an increase in the OTC rate was observed from 2003 to 2015, after which a plateau was reached with approximately 95 cases per year,

FIGURE 1



Distribution of Danish patients undergoing ovarian tissue cryopreservation (OTC) in relation to diagnosis and age groups. (**A**) Distribution of diagnoses in the cohort of patients undergoing OTC in Denmark from 1999 to 2020 (n = 1,186). (**B**) Distribution of diagnosis in relation to different age groups: <18 years, 18–29 years, and ≥30 years.

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corresponding to an annual incidence of 17–18 OTCs per million inhabitants.

Analysis of Usage of Cryopreserved Ovarian Tissue and Storage Time for the Entire Cohort

One girl and 116 women (n = 117; 10% of the entire cohort) returned for OTT between April 2003 and July 2020 mainly to restore or improve ovarian function to achieve pregnancy (n = 106) or, in a few cases, to restore hormone production (n = 10) or induce puberty (n = 1). The patients undergoing OTT were most commonly those who had breast cancer (38%) or lymphoma (20%). Table 1 shows the usage of cryopreserved ovarian tissue for the entire cohort (n = 1,186) who had undergone OTC from 1999 to 2020 (mean follow-up period of 8 years). In addition, it shows usage in relation to 5-year storage intervals. Out of all the women returning for OTT, most women (71%) had returned within the first 5 years after OTC. Only 6% of the patients had their tissue transplanted >10 years after OTC, all of whom had tissue cryopreserved in childhood or adolescence. The mean ages of the patients at OTC and OTT were 28.9 ±7 years and 33.2 ±6 years, respectively, resulting in a mean storage time of 4.3 years. Of the deceased patients (12%; n = 142/1,186), 86% died within the first 5-year period after OTC, and the mean ages of the patients at the time of OTC and death were 22.3 ± 9 years and 25.1 ± 10 years, respectively (Table 1). Seventy-two patients (6%; n = 72/1,186) had donated their cryopreserved tissue for research purposes (n = 70) or requested disposal (n = 2) at a mean age of 39.4 ± 5 years (Table 1).

Subgroup Analysis: Rates of OTT

In subgroup 1, the mean age of the patients was 24.6 ± 9 years and the mean follow-up period was 10.9 years. The overall return rate for OTT was 14% (n = 104/759) in subgroup 1 and 19% (n = 103/554) in subgroup 2 (including surviving patients aged >24 years). Table 2 shows usage stratified according to the diagnoses of the patients from subgroup 1 and OTT rates of the patients from subgroup 2. Individual OTT rates for each diagnosis were calculated for both subgroups, and OTT rates in the two subgroups varied markedly, especially for those diagnoses in which the patients had a low mean age at OTC and/or a high incidence of death (Table 2). In subgroup 2, the individual OTT rates were comparable among most diagnoses, ranging from 15% to 22%; lymphoma (18%), breast

TABLE 1

Use and storage time of cryopreserved ovarian tissue in the entire cohort (OTC from 1999 to 2020; n = 1,186).

Tissue use	No. of patients (%)	Age at OTC, years (mean ± SD) [range]	Age at OTT, death, or donation, years (mean ± SD) [range]	Use in relation to storage time			
				0–5 years (%)	5–10 years (%)	10–15 years (%)	> 15 years (%)
Transplanted	117 (10%)	28.9 ± 7 [9–42]	33.2 ± 6 [14–44]	71%	23%	5%	1%
Discarded	142 (12%)	22.3 ± 9 [0.5–39]	25.1 ± 10 [0.7–45]	86%	10%	4%	0%
Donated	72 (6%)	30.1 ± 4 [21–40]	39.4 ± 5 [31–50]	2%	55%	36%	7%
In storage (2020)	855 (72%)			54%	25%	17%	4%

Note: OTC = ovarian tissue cryopreservation; OTT = ovarian tissue transplantation.

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TABLE 2

Use of cryopreserved ovarian tissue according to diagnosis in patients with > 5 years follow-up (subgroup 1; n = 759).

Diagnosis	No. of patients	Age at OTC, years (mean ± SD)	Transplanted			Discarded		Donated	In storage (2020)	
			All patients No. (%)	Subgroup 2; Patients alive and > 24 years in 2020 (n = 554)	Storage time (years)	All patients No. (%)	Storage time (years)	All patients No. (%)	All patients No. (%)	Age in 2020, years (Mean ± SD)
Hematologic diseases										
- Lymphoma	159	23.6 ± 5.7	23 (15%)	23/131 (18%)	4.0 ± 3	23 (15%)	3.2 ± 4	15 (9%)	98 (61%)	33.2 ± 7
- Leukemia	60	15.5 ± 8.0	5 (8%)	5/34 (15%)	13.2 ± 3	14 (23%)	3.2 ± 4	1 (2%)	40 (67%)	29.1 ± 9
- Benign	33	15.9 ± 9.5	6 (18%)	6/17 (35%)	5.4 ± 4	3 (9%)	1.4 ± 1	1 (3%)	23 (70%)	24.1 ± 8
Breast cancer	259	31.5 ± 3.6	40 (15%)	40/222 (18%)	4.3 ± 2	37 (14%)	3.7 ± 3	41 (16%)	141 (55%)	40.3 ± 5
Sarcoma	73	18.1 ± 7.9	8 (11%)	7/33 (21%)	3.1 ± 2	25 (34%)	2.4 ± 3	1 (1%)	39 (53%)	27.3 ± 9
Gynecologic malignancies	66	27.7 ± 5.4	12 (18%)	12/55 (22%)	3.0 ± 2	9 (14%)	2.3 ± 2	5 (8%)	40 (60%)	36.7 ± 6
Neurologic malignancies	27	12.1 ± 9.3	1 (4%)	1/6 (17%)		10 (37%)	1.7 ± 1	1 (4%)	15 (55%)	21.6 ± 8
Gastrointestinal malignancies	21	29.1 ± 5.1	3 (14%)	3/11 (27%)	2.4 ± 1	10 (48%)	2.5 ± 2	1 (5%)	7 (33%)	37.2 ± 5
Other malignant diseases	1							1		
Systemic diseases	27	26.1 ± 6.5	4 (15%)	4/27 (15%)	5.5 ± 1	1 (4%)		3 (11%)	19 (70%)	35.8 ± 7
Genetic diseases	17	10.5 ± 6.7	0 (0%)	0/7 (0%)		0 (0%)		0 (0%)	17 (100%)	21.2 ± 7
Other benign diseases	16	21.1 ± 9.0	2 (13%)	2/11 (18%)		3 (19%)	1.3 ± 2	1 (6%)	10 (63%)	35.5 ± 7
Total	759		104/759 (14%)	103/554 (19%)		135/759 (18%)		71/759 (9%)	449/759 (59%)	

Note: Mean storage time was calculated in cases of three or more patients per group. Values are given as mean ± SD. OTC = ovarian tissue cryopreservation.

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cancer (18%), gynecologic malignancies (22%), sarcoma (21%), neurologic malignancies (17%), leukemia (15%), systemic diseases (15%), and other benign diseases (18%) (Table 2). Patients with benign hematologic diseases had the highest OTT rate of 35% followed by the patients with gastrointestinal malignancies who had an OTT rate of 27% (Table 2). In the patients with genetic diseases for whom OTC was primarily performed in childhood and adolescence, the OTT rates could not be assessed as most patients were still very young (10 out of 17 patients were <24 years of age) and none had returned for OTT (Table 2).

Subgroup Analysis: Mortality

Overall, 18% of the patients had died in subgroup 1 (n = 135/759). The highest relative mortality, according to the diagnosis, was reported for the patients with gastrointestinal malignancies (48%), neurologic malignancies (37%), sarcomas (34%), and leukemias (23%) (Table 2). The interval between OTC and death was the shortest for the patients with neurologic malignancies (mean 1.7 ± 1 year) and the longest for the patients with breast cancer (mean 3.7 ± 3 years).

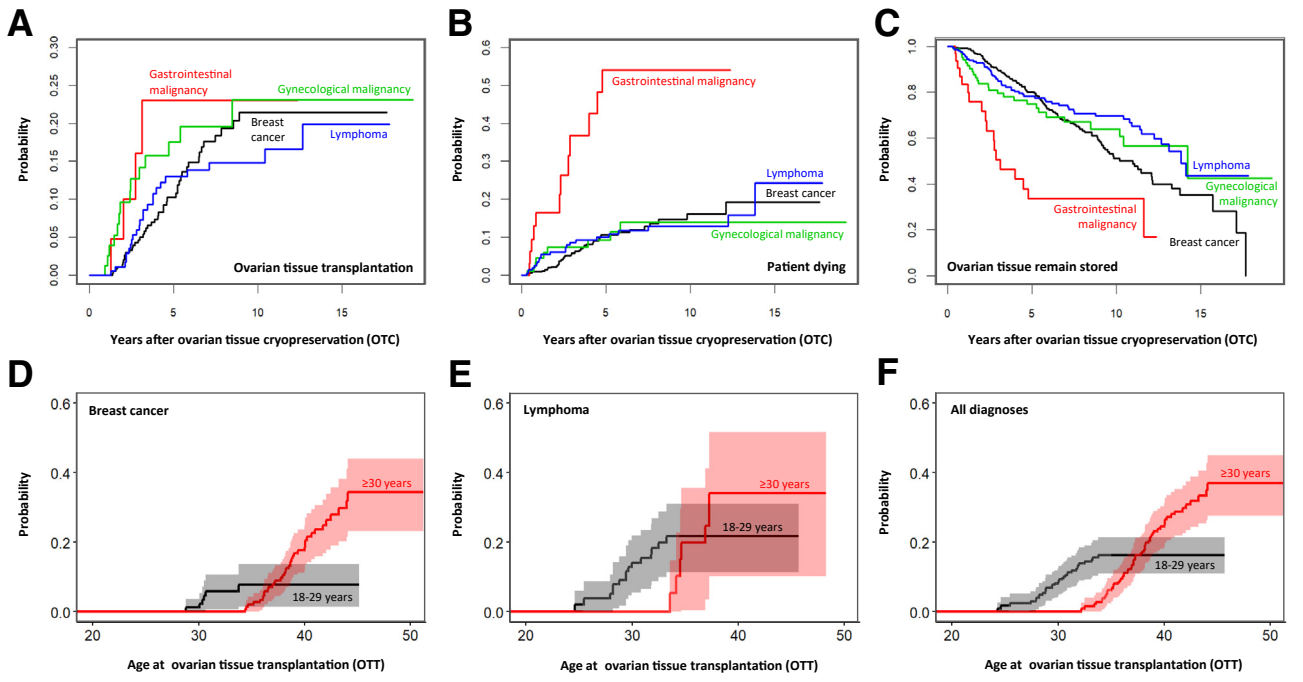
Subgroup Analysis: Tissue Still in Storage

Overall, 9% (n = 71/759) of the patients had donated their ovarian tissue for research in subgroup 1, and 59% (n = 449/759) still had their tissue in storage (Table 2). Of the 449 women with tissue in storage as of July 2020, half of them were aged >35 years and 27% were aged >40 years (n = 121), of whom 62% had breast cancer, 12% lymphoma, 9% gynecologic malignancies, 6% systemic diseases, and 3% leukemia. Breast cancer patients had the highest mean age at the time of OTC (31.5 ± 3.6 years) and at the end of follow-up (in 2020; 40.3 ± 5 years) as well as the highest tendency to donate their tissue for research (16%) followed by the patients with systemic diseases (11%), lymphomas (9%), and gynecologic malignancies (8%) (Table 2). All the patients with genetic diseases (mean age 10 years at OTC) still had their tissue in storage as none of the patients had died, donated, or undergone OTT. However, only 33% of the patients with gastrointestinal malignancies, 53% with sarcomas, 55% with neurologic malignancies, and 55% with breast cancer had their ovarian tissue in storage. This was mainly because of the high mortality rates associated with these diagnoses and the increased tendency to donate tissue in the breast cancer group (Table 2).

Usage Stratified According to the Selected Diagnoses

Kaplan-Meier plots were generated for the diagnoses for which the mean age of the patients was >18 years at the time of OTC, which included breast cancer, lymphoma, and gynecologic and gastrointestinal malignancies. The patients with other diagnoses were mostly young, and the follow-up period was too short to produce reliable plots. Figure 2A shows the likelihood of having OTT according to the selected diagnoses. Figure 2B shows the mortality risk, and Figure 2C shows the probability of the cryopreserved ovarian tissue remaining stored according to the diagnoses. Caution should be

FIGURE 2



The impact of diagnosis and age at ovarian tissue cryopreservation (OTC) on the use of cryopreserved ovarian tissue. (**A–C**) Probabilities were calculated for diagnoses in which the mean age of the patients was >18 years at OTC including breast cancer, lymphoma, gynecologic malignancy, and gastrointestinal malignancy. (**A**) The probability of the tissue being transplanted after OTC in women who were still alive in July 2020. (**B**) The probability of the patient dying. (**C**) The probability of the tissue remaining in storage after OTC. (**D–F**) The probability of OTT at a given age of the patient stratified on the age of the patient at OTC is depicted in patients with breast cancer (**D**), lymphoma (**E**), and for all diagnoses (**F**) for age groups 18–29 years (*black lines*) and ≥30 years (*red lines*). Graphs are depicted with 95% confidence intervals.

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made when interpreting the end of the Kaplan-Meier plots if large steps are present, as this usually reflects fewer observations/patients and broad 95% confidence intervals.

OTT Stratified According to the Age at OTC

The frequency of OTT increased with a higher age at the time of OTC. Only 4% of the girls aged <18 years at OTC (n = 10/242) had returned to undergo OTT, whereas 8% of the women aged 18–29 years at OTC (n = 44/533) and 15% of the women aged ≥30 years at OTC (n = 63/411) had undergone OTT. The duration of storage between OTC and OTT was the longest for the girls aged <18 years (mean 10.4 ± 4 years), whereas this duration was shorter but similar for the two older age groups (18–29 years: 3.7 ± 2 years; ≥30 years: 3.6 ± 2 years). Figure 2D to F shows the likelihood of having OTT according to the age groups 18–29 years and ≥30 years for the patients with breast cancer (Fig. 2D), lymphoma (Fig. 2E), and for all diagnoses (Fig. 2F), indicating that the patients aged ≥30 years at OTC were more likely to return for OTT. Probability plots for girls aged <18 years were not included, as the number of these patients who had undergone OTT was too small.

DISCUSSION

The Danish cohort is one of the largest and oldest cohorts of OTC, and the current study provides a 20-year insight into the use of cryopreserved ovarian tissue. This is the first study to stratify OTT rates in relation to the diagnosis and age of the patient at the time of OTC. Our findings highlight that the reporting of the OTT rate requires standardization to become a useful tool for fertility preservation programs and should ideally be reported according to diagnosis, age of the patient at OTC, and a defined follow-up period.

As expected, the return rate for OTT was higher in the subgroups with longer follow-up periods (subgroup 1: 14%; subgroup 2: 19%) when compared with the OTT rate for the entire cohort (10%). Assuming that approximately 22% of all women experience POI after OTC and gonadotoxic treatment in the present cohort (13), our results suggests that at least half of the women with POI return to make use of their cryopreserved tissue. However, the risk of POI differs significantly among diagnoses and usage in relation to the POI rate should be calculated according to the individual diagnoses. Moreover, further studies are needed to elucidate factors other than the POI rate that affect the OTT rates within the different diagnoses.

The return rates for OTT in the Danish cohort are the highest reported to date. Other fertility preservation programs have reported OTT rates ranging from 3.4% to 5.5% with mean follow-up periods of 6–7.6 years (4, 8, 11). The OTT rates in these studies were calculated by dividing the number of patients undergoing OTT by the total number of patients in the

entire cohort. However, on the basis of the current results, we argue that specific criteria for age at follow-up (>24 years), follow-up time (>5 years), and survival in relation to the diagnoses should be used to report and compare the return rates for OTT among centers and countries. This would facilitate identification of the patients who are most likely to benefit from OTC and OTT.

Breast cancer and lymphoma were the most frequent diagnoses associated with OTC and OTT, which was in line with results of previous studies (2, 7–10, 20). Interestingly, our study was unable to pinpoint any specific diagnosis for which the return for OTT differed markedly from the other groups. Our findings showed that the OTT rates were comparable (ranging from 15% to 22%) for most of the diagnoses when it was based on the number of surviving patients aged >24 years with >5 years of follow-up. Previous studies reported a low risk (9%–13%) of POI in breast cancer patients undergoing OTC (4, 9, 13). However, the return rate for OTT in the breast cancer patients (18%) was not markedly lower than that of the patients with other malignancies, like gynecologic (22%) and gastrointestinal (27%) malignancies, in which >30% of women have been reported to become menopausal (4). Thus, OTT rates appear to be related to factors other than the risk of POI after gonadotoxic treatment. The relatively high OTT rates in the patients with breast cancer might reflect that not only women developing POI return for their tissue but in addition women who wish to boost a low ovarian reserve without being menopausal.

Patients with leukemia undergoing bone marrow transplantation have the highest risk of POI (21, 22); however, whether or not patients with a previous diagnosis of leukemia should be offered OTT remains a matter of concern (4, 23–26). In our cohort, 15% of the patients with leukemia had returned for OTT, which was among the lowest OTT rates in the cohort, reflecting the contraindication for OTT. Including the five Danish patients, a total of 12 women with a previous diagnosis of leukemia have undergone OTT with no relapses reported to date, and four of them have conceived (3, 27–30). Nonetheless, the safety of the patient while considering OTT is of utmost importance and should always be evaluated carefully because of the risk of grafting leukemic cells.

Patients with benign hematologic diseases had the highest OTT rate (35%). Previous studies, including the study on four Danish patients, have reported 10 cases with β-thalassemia, sickle cell disease, aplastic anemia, and paroxysmal nocturnal hemoglobinuria undergoing OTT (10, 31–38). Remarkably, live births were achieved in seven patients, demonstrating that this patient group benefits from OTC and OTT with good efficacy.

Collectively, only 4% of the patients aged <18 years at the time of OTC in the Danish cohort had returned for OTT, and seven of these underwent OTT 10–15 years after OTC. The low return rate for OTT is in line with a recent study by Poirot et al. (39), who showed that only three patients had OTT out of 418 girls aged <15 years undergoing OTC between 1998 and 2018. It will probably take at least another 10 years to obtain reliable OTT rates for these young girls.

Stratified age analysis showed that more women aged ≥30 years at OTC had returned for OTT compared with younger women, which would logically be related to the follow-up time. Kaplan-Meier curves showed that over time women aged ≥30 years at OTC were more likely to return for OTT than the women aged 18–29 years at OTC. These findings may reflect that the older age group (≥30 years) had already reached an age at which pregnancy becomes more desirable, and they might in addition be more likely to experience infertility after gonadotoxic treatment because of their advanced age and reduced ovarian reserve at the time of OTC. However, this trend differed between some diagnoses (breast cancer and lymphoma), which indicates that different treatment regimens and risks of POI affect the return for OTT in the different diagnosis and age groups. Interestingly, the time period from OTC to OTT was similar in women aged ≥30 years at OTC (3.6 years) and women aged 18–29 years at OTC (3.7 years), which indicates that women in their 20s at OTC do not return later for OTT than women who were in their 30s at OTC. This finding is of clinical interest with regard to the planning of follow-up and after-cancer reproduction in these patients. However, further studies with longer follow-up are needed to elucidate this aspect and confirm these findings. Additionally, further studies are needed to reveal the efficacy of OTT in patients of advanced maternal age, because studies have reported very poor reproductive outcomes after OTT in women who were >35 years old at the time of OTC (7, 40). Such studies are especially important in the context of breast cancer patients who had the highest mean age at OTC in the Danish cohort.

Overall, 12% of the Danish patients had died, which is in line with results from previous studies (4, 9, 14). In the Danish cohort, 86% of the deceased patients died within the first 5-year period after OTC, probably reflecting the severeness of their diseases. The highest mortality rate was found in the patients with gastrointestinal and neurologic malignancies and sarcomas, which corroborates results from previous studies (4, 14).

Finally, our 20-year retrospective analysis revealed that a fairly large proportion of the Danish patients might not return and make use of their cryopreserved ovarian tissue. The main reason was probably that a large proportion of the women conceived without the use of their frozen tissue because of a fairly low risk of POI in the largest patient groups (i.e., breast cancer and lymphoma) (13). In a previous Danish study, 72% of women who tried to become pregnant after OTC and oncologic treatment succeeded (13). However, long-term follow-up studies are needed to assess the fertility and long-term risk of POI in OTC patients, highlighting the importance and need for regular after-cancer follow-up of these patients by fertility specialists to identify their potential needs for using the cryopreserved ovarian tissue (41). Such measures could potentially increase the return rate for OTT and provide much needed insight into after-cancer reproduction.

One limitation of the current analysis was that the follow-up period was too short to properly assess the return rates in the patients who had undergone OTC in childhood and adolescence. Furthermore, stratified analysis according to the diagnoses was based on a small number of patients, and solid conclusions could not be made. Another limitation was that the status of all women in relation to natural

conceptions was not available, and this is of importance as a number of women had already fulfilled their intended family size at the time of follow-up. From a socioeconomic aspect, the OTC procedure (including oophorectomy, storage, and OTT) is covered by the Danish public health care services, which hinders a direct comparison of the results of our study with those of other studies from countries with private medical care. Finally, the status of ovarian function and reproductive outcomes were not included; thus, the efficacy of OTT could not be evaluated.

CONCLUSION

The return rate for OTT among women in the Danish cohort is the highest reported to date, and almost one in five patients who reached an age at which childbearing becomes of interest returned for OTT. Our findings showed that the OTT rate was dependent on the diagnosis, age of the patient at OTC, and follow-up time, which calls for new measures for reporting and comparing the OTT rates within cohorts and among centers. Patients who have not yet made use of their cryopreserved tissue because of young age at OTC may still return for OTT, and long-term follow-up is still needed to reveal the "true" return rates for especially the young girls undergoing OTC.

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Utilización de tejido ovárico criopreservado en la cohorte danesa de preservación de fertilidad.

Objetivo: Evaluar el uso de tejido ovárico criopreservado en la cohorte danesa de preservación de fertilidad.

Diseño: Estudio retrospectivo de cohortes.

Entorno: Hospitales universitarios y clínicas de fertilidad.

Paciente(s): Se realizó criopreservación de tejido ovárico (OTC) en 1,186 mujeres danesas entre 1999 y 2020, de las cuales 117 se sometieron posteriormente a trasplante de tejido ovárico (OTT). El subgrupo 1 incluyó a 759 pacientes con un periodo de seguimiento $>$ 5 años. De ellas, las tasas de OTT fueron posteriormente analizadas para aquellas pacientes que estaban vivas y con edad $>$24 años en julio de 2020 (subgrupo 2; n=554).

Intervención(es): OTC y OTT.

Medida(s) del resultado principal(es): OTT, fallecimiento, donación de tejido.

Resultado(s): En el subgrupo 1, el 14% de las pacientes se habían sometido a OTT, el 18% habían fallecido, el 9% habían donado su tejido para investigación y el 59% todavía tenían su tejido almacenado. En el subgrupo 2, 19% se habían sometido a OTT y, para la mayoría de diagnósticos las tasas de OTT oscilaban del 15 al 22%, teniendo las enfermedades hematológicas benignas la mayor tasa de OTT (35%%). Respecto a la totalidad de la cohorte, el análisis estratificado por edad indicó que las mujeres de edad $\geq$ 30 años tenían mayor probabilidad de regresar para OTT que las mujeres con edades entre 18 y 29 años en el momento de la OTC; los tiempos medios de almacenamiento fueron de 3.7 y 3.6 años respectivamente. Únicamente el 4% de las mujeres con edad $<$ 18 años en el momento de la OTC se habían sometido a OTT.

Conclusión(es): Las tasas de OTT dependían del diagnóstico, edad en el momento de la OTC y tiempo de seguimiento. Son necesarios criterios específicos para informar y comparar las tasas de OTC. Seis de cada 10 pacientes todavía tienen su tejido criopreservado almacenado y se necesita más tiempo de seguimiento, especialmente para las mujeres más jóvenes.