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Ovarian tissue cryopreservation and transplantation among alternatives for fertility preservation in the Nordic countries – compilation of 20 years of multicenter experience

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Key words

Cancer, female, fertility preservation, oocytes, ovarian tissue cryopreservation, ovarian transplantation

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Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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Abstract

Introduction. The aim of this study is to report the current status of ovarian tissue cryopreservation among alternatives for fertility preservation in the Nordic countries. **Material and methods.** A questionnaire was sent to 14 Nordic academic reproductive centers with established fertility preservation programs. It covered fertility preservation cases performed up to December 2014, standard procedures for ovarian tissue cryopreservation and oocyte cryopreservation and reproductive outcomes following ovarian tissue transplantation. **Results.** Among the Nordic countries, Denmark and Norway practice ovarian tissue cryopreservation as a clinical treatment (822 and 164 cases, respectively) and their programs are centralized. In Sweden (457 cases), ovarian tissue cryopreservation is practiced at five of six centers and in Finland at all five centers (145 cases). Nearly all considered ovarian tissue cryopreservation to be experimental. In Iceland, embryo cryopreservation is the only option for fertility preservation. Most centers use slow-freezing methods for ovarian tissue cryopreservation. Most patients selected for ovarian tissue cryopreservation were newly diagnosed with cancer and the tissue was predominantly retrieved laparoscopically by unilateral oophorectomy. Only minor complications were reported. In total, 46 women have undergone ovarian tissue transplantation aiming at recovering fertility, 17 healthy children have been born and several additional pregnancies are currently ongoing. Whenever patients' clinical condition is permissive, oocyte cryopreservation after hormonal stimulation is preferred for fertility preservation. Between 2012 and 2014, a smaller proportion of females have undergone fertility preservation in the Nordic centers, in comparison to males (1:3). **Conclusions.** Overall, ovarian tissue cryopreservation was reported to be safe. Slow freezing methods are still preferred. Promising

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results of recovery of fertility have been reported in Nordic countries that have initiated ovarian tissue transplantation procedures.

Abbreviations: OTC, ovarian tissue cryopreservation.

Introduction

Data from the Association of Nordic Cancer Registries indicate that in the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden), approximately 145 000 individuals are diagnosed with cancer each year. About 10 000 of these are children, adolescents or young adults of reproductive age (1). Fertility preservation has gained increased acceptance in reproductive medicine and many centers worldwide have established programs for this service. Young individuals diagnosed with cancer are the most representative patients with clear indications for fertility preservation, owing to the recognized highly toxic effects of chemotherapy and high-dose radiation on the gonads and subsequent development of infertility as a result (2). As improvements in cancer therapy are resulting in increasing numbers of long-term survivors, all quality-of-life aspects, including the preservation of fertility, have become of major importance. International guidelines for fertility preservation have been provided (3,4) and these have had an impact on clinical practice in the medical community. Hence the number of international reports and publications, including preclinical, clinical and epidemiological research on fertility preservation for adults and children is constantly increasing (5).

For female patients, methods for fertility preservation have been developed and are currently classified into clinically established methods such as cryopreservation of embryos and oocytes, whereas ovarian tissue cryopreservation (OTC) is still considered experimental by international collaborative work groups, such as those recently organized by the American Society of Clinical Oncology (4) in 2013 and by the American Society of Reproductive Medicine (ASRM) (6) in 2014. In Europe, large series of women who have undergone OTC have been reported (7,8). When combining reported live births in these series, 28 women of 80 who underwent ovarian tissue transplantation in Belgium, Denmark, Germany and Spain, are indicative of a promising 35% success rate with additional pregnancies ongoing (7–9). Worldwide, however, the lack of an international register means that the number of transplantations performed is not known, as many centers have not yet reported their results.

The aim of our study was to collect and report data from the Nordic countries with regard to the development of programs for fertility preservation for female

patients. We have focused on reporting activities in OTC and transplantation procedures and also oocyte cryopreservation for fertility preservation. In all the Nordic countries, standards of care include national health insurance programs, which cover infertility investigation and subsequent performance of assisted reproductive techniques, with only modest differences in regulations regarding access to such medical care across the countries. In the Nordic countries, OTC is currently restricted to fertility preservation programs at academic reproductive centers that belong to large university hospitals. So far, the only Nordic center that has reported reproductive outcomes of their OTC program is the group from Rigshospitalet University Hospital, Copenhagen, representing three clinics covering the entire Danish population (9,10). In addition, a few centers have reported successful single cases (11,12), but the overall activity in the Nordic countries remains, until now, elusive and has not been reported.

Material and methods

All Nordic university hospitals with established reproductive medicine centers that have initiated programs for fertility preservation indicated by medical reasons and that might practice OTC were identified for this survey ($n = 14$). Thirteen of the reproductive medicine centers belong to their university hospitals. The remaining center (ART Medica, Reykjavik, Iceland) is a privately run clinic associated with Landspítali University Hospital of Reykjavik, for teaching activities. Additional private reproductive centers that perform elective oocyte cryopreservation were not considered. Clinicians responsible for fertility preservation programs at the centers were requested to respond to a questionnaire developed for this study (see Appendix S1). The questionnaire, developed by K.R.W.,

Key Message

Ovarian tissue cryopreservation is practiced at most Nordic Reproductive Medicine centers and it was reported as safe and effective. Recovery of fertility by ovarian tissue transplantation has been achieved in several centers that have initiated transplantation procedures.

T.T. and C.Y.A., concerned historical development of fertility preservation programs including legal and technical aspects, initiation of fertility preservation by OTC and oocyte cryopreservation and cases performed until 31 December 2014, methodology used and changes over time in addition to clinical characteristics of patients and standard procedures for OTC, complications, number of cases of ovarian tissue transplantation performed, and reproductive outcomes. The annual numbers of both female and male fertility preservation cases at the centers during the period 2012–2014 was also requested, to obtain a clinical context and comparator to female fertility preservation and OTC within the fertility preservation programs.

Submitted data, with last entry 30 October 2015 were primarily compiled by K.R.W. All 14 centers replied (100% response). The procedures were in accordance with the ethical standards of the responsible local or national committee on human experimentation and with the Helsinki Declaration of 1975, revised in 1983. Ethics approval for the review of medical records and for these analyses was granted by the Regional Ethics Committee in Stockholm (Dnr 2011/1758-31/2 and Amendment 2014/1825-32) and by local ethics committees.

Results

Historical, technical and legal aspects

The Sahlgrenska University Hospital in Gothenburg pioneered OTC by offering it to three women aiming at fertility preservation in 1995; the women underwent this procedure at their Department of Obstetrics and Gynecology. The indication was potentially gonadotoxic treatment of malignancy – one woman with breast cancer and two with Hodgkin's lymphoma. Tissue was cryopreserved according to the method developed by Gosden *et al.*, using dimethylsulfoxide and sucrose as cryoprotectants and a slow-freezing protocol (13). In 1999 OTC was initiated at both Rigshospitalet University Hospital in Copenhagen and at Karolinska University Hospital in Stockholm. The protocols established at these centers included slow-freezing methods using ethylene glycol and sucrose (14), and propanediol and sucrose (15), respectively. Table 1 shows the methods that are currently practiced at each center. Two centers have changed their methods for OTC over time. Further research at the Karolinska Institute allowed the development of vitrification methods for cryopreservation of ovarian tissue (16,17) and at Karolinska University Hospital tissue retrieved for fertility preservation was cryopreserved by vitrification in 2009–2012. Today, half of the ovarian tissue retrieved is cryopreserved by slow-freezing and the remaining half by vitrification. Overall, the

slow-freezing methods are still preferred and practiced at all centers. Vitrification of ovarian tissue was also tested during a 2-year period at Tampere University Hospital (2009–2011), but the group then continued with slow freezing for OTC.

Two Nordic countries have national centralized programs established for OTC. In Denmark, a program was established at Rigshospitalet, after approval by the Ministry of Health in Copenhagen and Frederiksberg (J/KF/01/170/99). Similarly, in Norway, the Ministry of Health and Care services centralized OTC activity to Oslo University Hospital in 2004. In both countries, the performance of OTC is approved as a clinical treatment.

In Finland and Sweden, fertility preservation programs have been developed at university hospitals that provide healthcare to large regions/counties. One center in Sweden still does not practice OTC. There is no established agreement in the categorization of OTC as a clinical or experimental option for fertility preservation (Table 1).

Regarding oocyte cryopreservation, the first Nordic center to perform this procedure for fertility preservation was the Center for Reproduction at Uppsala University Hospital in 1994. Several centers included oocyte cryopreservation within their programs for fertility preservation during the 1990s. Slow-freezing methods were performed for about 13 years until the introduction of oocyte vitrification at the clinics, which commenced in 2007. Currently, commercial and kit-based methods for vitrification with closed systems are used at all centers.

Most centers prefer the option of oocyte cryopreservation for fertility preservation in adult women rather than performing OTC, if time is available and the clinical condition of the patient allows ovarian stimulation and oocyte retrieval. In Iceland, the only method practiced for fertility preservation is the freezing of embryos, however, a partnership collaboration has been established with Karolinska University Hospital in Stockholm for patients interested in oocyte cryopreservation after hormonal stimulation (Table 1).

All procedures for female fertility preservation are reimbursed in agreement with national healthcare policies at all centers.

Clinical characteristics of patients and standard procedures for OTC

Patient characteristics and indications for OTC at the centers performing this option for fertility preservation are presented in Table 2. All centers have included adult women, but restricted OTC to women younger than 40 years of age. Exceptionally, OTC procedures have been offered to women above that age. Common indications for adult women include breast cancer, Hodgkin's disease,

Table 1. Ovarian tissue cryopreservation (OTC) and additional fertility preservation options for females at Nordic centers; characteristics of the program, date of initiation of OTC, current methods, reimbursement status and storage limits are presented.

Center	OTC started (year)	Type of program	Other methods available for female fertility preservation	Procedures reimbursed	Method for OTC	Limit for storage	Counseling provided	OTC Clinical vs. Experimental
Denmark Copenhagen Rigshospitalet University Hospital Finland	1999	National centralized	Embryo, oocyte cryopreservation since 2006	Yes	Slow freezing, ethylene glycol, sucrose and HSA (14)	No	RMS	Clinical since 1999
Kuopio University Hospital	2000 ^a	Regional	Oocyte cryopreservation	Yes	Slow freezing	No	Ob/Gyn, Oncologist	Clinical since 2007
Oulu University Hospital	2008	Regional	Oocyte cryopreservation since 2009	Yes	Slow freezing, ethylene glycol, sucrose and HSA (14)	Female age 40	RMS, Oncologist	Experimental
Helsinki University Hospital	1999	Regional	Embryo, oocyte cryopreservation since 2010	Yes	Slow freezing ^c	No	Ob/Gyn	Experimental
Tampere University Hospital	2000	Regional	Oocyte cryopreservation	Storage fee	Slow freezing	No	Ob/Gyn, RMS	Clinical since 2000
Turku University Hospital	2002 ^b	Regional	Embryo, oocyte cryopreservation	Yes Storage fee after 6 years	Slow freezing	No	RMS, oncologist	Clinical for adults since 2014. Currently there is not any investigational protocol for children and therefore OTC is not available for girls
Iceland Reykjavik ART Medica	-	National	Embryo freezing only	Yes	-	5 years (Embryos)	Ob/Gyn	OTC not approved
Norway Oslo University Hospital	2004	National centralized	Oocyte cryopreservation since 2012	Yes	Slow freezing, ethylene glycol, sucrose and HSA since 2008 (14) ^d	Female age 45	RMS	Clinical since 2004
Sweden Gothenburg, Sahlgrenska University Hospital	1995	Regional	Embryo, oocyte cryopreservation	Yes	Slow freezing with DMSO (13)	No	RMS	Clinical for adults, experimental for girls
Linköping University Hospital	2002	Regional	Embryo, oocyte cryopreservation	Yes	Slow freezing with propanediol and sucrose (15)	No	RMS, Oncologist	Clinical
Örebro University Hospital	-	Regional	Embryo and oocyte cryopreservation since 2006	Yes	-	No	Ob/Gyn, RMS, Oncologist	OTC not approved

Table 1. Continued

Center	OTC started (year)	OTC (year)	Type of program	Other methods available for female fertility preservation	Procedures reimbursed	Method for OTC	Limit for storage	Counseling provided	OTC Clinical vs. Experimental
Uppsala University Hospital	2000	Yes	Regional	Embryo, oocyte cryopreservation	Yes	Slow freezing, ethylene glycol, sucrose and HSA since 2011 (14) ^d	No	RMS	Experimental
Malmö, Skåne University Hospital	2001	Yes	Regional	Embryo, oocyte cryopreservation	Yes	Slow freezing, ethylene glycol, sucrose and HSA (14)	No	Ob/Gyn, Oncologist, RMS	Clinical
Stockholm, Karolinska University Hospital	1999	Yes	Regional	Embryo, oocyte cryopreservation	Yes	Slow freezing with propanediol and sucrose (15), vitrification since 2009 (16,17)	No	Ob/Gyn, RMS	Experimental

HAS, human serum albumin; Ob/Gyn, specialist in Obstetrics and Gynecology; RMS, Ob/Gyn subspecialist in Reproductive Medicine.

^aOTC was practiced at Kuopio between 2000 and 2004. The tissue has been sent to Tampere for performance of OTC and storage since 2005.

^bOTC was practiced at Turku between 2002 and 2004. The procedures were initiated in 2014 in collaboration with Tampere University Hospital, where OTC is currently performed and the tissue stored.

^cOTC in collaboration with the Family Federation of Finland's fertility clinics that currently perform the cryopreservation procedures.

^dCenters that changed method from initial propanediol and sucrose.

lymphoma, sarcoma, and gynecological cancer. Several centers have performed OTC for children, in most cases indicated by malignancies (hematological cancer, sarcoma, Hodgkin's lymphoma, central nervous system malignancy), but OTC has also been performed in some centers in connection with benign conditions such as Turner syndrome.

Unilateral oophorectomy is performed in most centers and none have reported any severe complications. Infection screening is routinely performed at all centers, according to European standards recommended by the European Union Tissues and Cells Directives.

Patients who have undergone oocyte cryopreservation

In Table 3 the most common indications for fertility preservation by oocyte cryopreservation are presented. Although no absolute numbers according to diagnosis were requested, nine out of 14 centers reported that women with breast cancer are the largest patient group to undergo these treatments, followed by women with hematological malignancies (Table 3). Several centers have included stimulation protocols adapted for breast cancer in their fertility preservation programs (18,19).

Only rarely have women older than 40 years undergone fertility preservation by oocyte cryopreservation at Nordic clinics (Table 3). Most programs follow the age limits recommended for assisted reproductive technology in their countries as regards the performance of female fertility preservation, i.e. fertility preservation procedures can only be offered to women within the age limits for national healthcare policy regulated and reimbursed assisted reproductive technology, which in Sweden is up to a female age of 40 years.

Ovarian tissue transplantation and clinical outcomes

Table 4 presents a summary of ovarian tissue transplantation activities in the Nordic countries and the results obtained among women who have requested transplantation of the tissue to recover fertility.

Several centers have initiated transplantation procedures. In some cases, including seven women treated at Rigshospitalet University Hospital and two women treated at Karolinska University Hospital, the indication for reimplantation was aimed at the relief of climacteric symptoms. In one girl treated at Rigshospitalet University Hospital, the primary indication was puberty induction (20).

With regard to women with fertility wishes, the ovarian tissue transplantation procedures have been successful at several centers, with the greatest experience of

Table 2. Clinical characteristics of patients (total $n = 1608$) included in fertility preservation programs that involve ovarian tissue cryopreservation (OTC) in Nordic centers, standard routines and complications registered.

Center practicing OTC	No. of OTC patients	Age range (n)	Common diagnoses in adults	Common diagnoses in children	Tissue retrieved	Infection screening (year initiated)	Complications registered
Denmark							
Copenhagen, Rigshospitalet University Hospital	822	18–38 (594) 13–17 (153) 0.6–12 (76)	Breast cancer, Hodgkin's lymphoma, sarcoma	Hematological malignancies, sarcoma, CNS malignancy	Unilateral oophorectomy	Yes	None
Finland							
Helsinki University Hospital	71	<18 (71)	–	Hematologic malignancies	Ovarian biopsies	Yes (2005)	None
Kuopio University Hospital	10	18–30 (10)	Sarcoma, gynecological cancer, Hodgkin's lymphoma	–	Individualized from ovarian biopsies to unilateral oophorectomy	Yes (2007)	None
Oulu University Hospital	9	18–34 (9)	Hodgkin's lymphoma, breast cancer, lymphoma	–	Ovarian biopsies	Yes (2008)	None
Tampere University Hospital	70	17–36 (63) ^a 15–16 (7)	Hodgkin's lymphoma, breast cancer, sarcoma	Hodgkin's lymphoma, sarcoma	Ovarian biopsies	Yes (2003)	Minor (bleeding)
Turku University Hospital	5	24–32 (4) <12 (1)	Gynecological cancer, other	Cancer of the nervous system	Individualized from ovarian biopsies to unilateral oophorectomy	Yes (2002)	None
Norway							
Oslo University Hospital	164	18–36 (135) 10–17 (29)	Breast cancer, lymphoma, sarcoma	Lymphoma, sarcoma, hematological malignancies	Unilateral oophorectomy	Yes (2004)	None
Sweden							
Gothenburg Sahlgrenska University Hospital	35	18–43 (34) 15–17 (1)	Hodgkin's lymphoma, breast cancer, gynecological cancer	Neuroblastoma, neural	Unilateral oophorectomy	Yes (2003)	Minor (bleeding)
Linköping University Hospital	24	17–35 (4) ^a 3–13 (20)	Breast cancer, other	Turner syndrome	Ovarian biopsies	Yes (2002)	None
Uppsala University Hospital	25	18–38 (22) 12–16 (3)	Breast cancer, Hodgkin's lymphoma, gynecological cancer	Turner syndrome, ovarian teratoma, vaginal cancer	Unilateral oophorectomy	Yes (2000)	None
Malmö Skåne University Hospital	72	17–39 (69) ^a <17 (3)	Breast cancer	Malignancies	Unilateral oophorectomy	Yes (2001)	None
Stockholm Karolinska University Hospital	301	18–39 (188) 3–17 (113)	Breast cancer, lymphoma, sarcoma, gynecological cancer	Leukemia, cancer of the nervous system, Turner syndrome	Individualized from ovarian biopsies to unilateral oophorectomy	Yes (2000)	Minor (bleeding)

^aPatients of 17 years of age were classified as children at most centers, whereas they were grouped with the adults at three centers (Tampere, Linköping and Malmö).

Table 3. Oocyte cryopreservation for fertility preservation of females at 14 Nordic centers (total $n = 455$); current methods, date of initiation and clinical characteristics of patients are presented.

Centers performing oocyte cryopreservation for fertility preservation	Year of start: slow freezing/vitrification	Method preferred, OTC vs. oocyte cryopreservation	No. of cases of oocyte cryopreservation	Age range	Common indications
Denmark					
Copenhagen Rigshospitalet University Hospital	2006/2010	Both available	20	32–43	Breast cancer, ^a genetic conditions, hematological
Finland					
Helsinki University Hospital	2010/2012	Oocyte cryopreservation	12	18–38	Lymphoma, breast cancer
Kuopio University Hospital	–/2012	Oocyte cryopreservation	5	13–30	Cancer, need of stem cell transplantation
Oulu University Hospital	2009/2012	Oocyte cryopreservation	3	15–40	Hodgkin's lymphoma, hematological benign diseases
Tampere University Hospital	2007/2011	Both available	5	17–32	Lymphoma, breast cancer, ovarian tumor
Turku University Hospital	–/2012	Oocyte cryopreservation	5	22–32	Breast cancer, ^a benign premature ovarian insufficiency
Iceland					
Reykjavik Art Medica	–/–	Oocyte cryopreservation in collaboration with Karolinska Hospital since 2014	2	33–35	Breast cancer ^a
Norway					
Oslo University Hospital	–/2014	OTC	0	–	–
Sweden					
Gothenburg Sahlgrenska University Hospital	1995/2010	Oocyte cryopreservation if time available	74	17–40	Breast cancer, ^a Hodgkin's lymphoma, cervical cancer
Linköping, University Hospital	2007/2013	Oocyte cryopreservation if time available	28	16–35	Breast cancer, ^a other malignancies, need of stem cell transplantation
Örebro, University Hospital	2006/2012	Oocyte cryopreservation	12	19–35	Breast cancer, ^a lymphoma, other malignancies
Uppsala, University Hospital	1994/2008	Oocyte cryopreservation	42	17–38	Breast cancer, ^a need of stem cell transplantation, other malignancies
Malmö Skåne University Hospital	–/2013	Both available	25	21–39	Breast cancer ^a
Stockholm, Karolinska University Hospital	1999/2007	Oocyte cryopreservation	222	15–42	Breast cancer, ^a hematological malignancies

^aAt nine of the 14 centers, breast cancer was reported as the most common cause for fertility preservation by oocyte cryopreservation.

transplantation of frozen and thawed ovarian tissue at Rigshospitalet in Copenhagen, where currently 14 children have been born to women who regained fertility through these procedures. Additionally, there are currently a few ongoing pregnancies at the time of preparing this report.

Age limits for reimplantation of ovarian tissue have been considered at most of the centers, and some centers agree on the fact that the tissue should not be transplanted to postpone the natural menopausal age (Table 4).

Fertility preservation for females and males

The number of young patients (both male and female) that were referred for fertility preservation at the centers

during the last 3 years was also investigated. The data are presented in Table 5. The numbers of both women and men who undergo fertility preservation are increasing at all centers, although the number of females who have undergone fertility preservation is still small in comparison with that of males who have banked frozen sperm, approximately one in three.

Discussion

The focus of this survey was to collect and report data on female fertility preservation activities through OTC and ovarian transplantation procedures in the Nordic countries. Furthermore, data on additional fertility preservation options for females such as oocyte cryopreservation

Table 4. Transplantation of frozen–thawed ovarian tissue at Nordic centers; clinical indications for ovarian tissue transplantation, cases with fertility wishes and reproductive outcomes.

Centers performing OTC	Recommended age limit for reimplantation	Indications for reimplantation of ovarian tissue	Women wishing reimplantation		Transplantations performed (n)	Orthotopic/Heterotopic	Cases wishing for fertility (n)	Results
			for reimplantation (n)	wishing reimplantation (n)				
Denmark								
Copenhagen Righospitalet University Hospital	No age limit but should not take place beyond usual age of menopause	For fertility and for treatment of climacteric symptoms/puberty induction	41	0	53 (retransplantation in some patients)	Preferred orthotopic; performed both	32	14 children born (ref. 9)
Finland								
Kuopio University Hospital	Premenopausal age	For fertility and for treatment of climacteric symptoms	0	0	0	–	–	–
Oulu University Hospital	40	Only for fertility	1	1	1	Orthotopic	1	IVF/ICSI and ET in one case. No pregnancy
Helsinki University Hospital	Premenopausal age	Only for fertility	0	0	0	–	–	–
Tampere University Hospital	Limit for storage to approx. 43 years of age	Only for fertility	5	3	3	Orthotopic	3	Three patients underwent IVF/ICSI and ET. One of the treatments resulted in an ongoing clinical pregnancy (week 20)
Norway								
Turku University Hospital	No age limits	Only for fertility	0	0	0	–	–	–
Oslo University Hospital	45	For fertility and for treatment of climacteric symptoms	4	2	2	Orthotopic	4	One woman conceived spontaneously and delivered a child. One woman conceived after IVF/ICSI and ET and delivered a child (ref. 11)
Sweden								
Gothenburg Sahlgrenska Univ. Hospital,	40	Only for fertility	2	1	1	1	1	One spontaneous clinical pregnancy ongoing (week 8)
Linköping University Hospital	40	–	0	0	0	–	–	–
Uppsala University Hospital	Before 45 years of age	Only for fertility	1	1	1	Heterotopic	0	Relief of climacteric symptoms
Malmö Skåne University Hospital	No age limits	Also possible for treatment of climacteric symptoms but not for postponing menopause	2	2	2	Preferred orthotopic; performed both	2	No pregnancies

Table 4. Continued

Centers performing OTC	Recommended age limit for reimplantation	Indications for reimplantation of ovarian tissue	Women wishing reimplantation (n)	Transplantations performed (n)	Orthotopic/Heterotopic	Cases wishing for fertility (n)	Results
Stockholm Karolinska University Hospital	45–46 years of age	For treatment of climacteric symptoms but not for postponing menopause	6	9 (retransplantation in some patients)	Preferred orthotopic; performed both	4	One woman wished to conceive spontaneously. Two women underwent IVF/ICSI and ET. The treatments resulted in one live birth (ref. 12) and one ectopic pregnancy, which was treated medically. One woman has recently undergone OTT.
Total cases			62	72		47	17 children born, two ongoing pregnancies

ET, embryo transfer; IVF/ICSI, in vitro fertilization/intracytoplasmic sperm injection; OTC, ovarian tissue cryopreservation.

were also requested, as well as the centers' preferences as regards to these methods. Our results indicate that OTC has been practiced on a large scale and for many years at certain Nordic centers. Most OTC procedures have been carried out to preserve fertility in women and girls with malignancies and to a minor degree have also been offered to girls with benign conditions such as Turner syndrome. Surgical retrieval of ovarian tissue is considered to be safe as self-reported by the centers; no major complications were recorded, although it should be noted in this context that there may be recall bias. Importantly, our data are in agreement with results from several European groups that have reported OTC to be a safe activity in female programs for fertility preservation (7,21,22).

The efficacy of ovarian tissue transplantation procedures for regaining fertility is also proven in this study, as the procedures have resulted in successful pregnancies and healthy children, which even occurred at centers that had only recently initiated transplantation of ovarian tissue and that did not have any previous experience of this type of surgery. Our findings are also in line with previous data (7–9), and are encouraging for suitable centers that have not yet implemented this service with OTC and reimplantation.

Most of the centers that do not have national centralized programs reported that they would prefer the option of oocyte cryopreservation for female fertility preservation, rather than OTC, if a woman's condition allowed hormonal stimulation and time was available. An important argument for this was that oocyte cryopreservation is today considered as an established clinical option for fertility preservation (4,23) and reproductive medicine specialists are familiar with the procedures. At such centers, OTC came as a second option for adult women, or in cases of unwanted hormonal stimulation or when there is a lack of time. Notably, only a few pregnancies have been reported in women with cancer based on vitrification of mature oocytes (24). Hence, the efficacy of this approach needs to be evaluated after actual clinical experience. The OTC procedure is clearly preferred as a first-line procedure for young girls and prepubertal patients at all centers, which is in line with international recommendations (4,25).

Our finding of an increasing number of patients referred for fertility preservation at all university-based centers indicates that oncologists and other specialists treating young people for malignant and chronic diseases are increasingly becoming aware of the fertility concerns of their patients when planning gonadotoxic treatments. However, our data raise implications as regards to access and performance of fertility preservation, which seems to be more restricted for women than for men, as the number of women referred for fertility preservation at centers

Table 5. The number of patients referred for fertility preservation is increasing at all centers; in most centers, the number of males is several times higher than the number of females.

Centers performing fertility preservation for female and male patients	Females; cases of fertility preservation last three consecutive years (n)			Males; cases of fertility preservation last three consecutive years (n)		
	2012	2013	2014	2012	2013	2014
Denmark						
Copenhagen Rigshospitalet University Hospital	67	65	73	Not centralized ^a		
Finland						
Kuopio University Hospital	3	5	3	15	10	11
Oulu University Hospital	–			32	23	33
Helsinki University Hospital	5	7	10	50	60	70
Tampere University Hospital	8	6	7	25	38	22
Turku University Hospital	3	0	3	14	36	23
Iceland						
Reykjavik ART Medica	2	2	2	7	6	14
Norway						
Oslo University Hospital	20	13	19	176	156	154
Sweden						
Gothenburg Sahlgrenska University Hospital	9	26	33	77	79	100
Linköping University Hospital	3	7	11	40	50	60
Örebro University Hospital	4	6	4	15	19	26
Uppsala University Hospital	21	22	17	81	74	76
Malmö Skåne University Hospital	15	20	22	82	89	100
Stockholm Karolinska University Hospital	125	109	116	125	160	162
Total cases	285	288	321	739	800	871

Data shown include patients referred per year for fertility preservation at the Nordic centres between 2012 and 2014.

^aIn Denmark, freezing and banking of sperm is not centralized at Rigshospitalet University Hospital and is available at many centres.

that receive referrals for both genders was very low in comparison with the respective numbers for men.

This can to some extent be explained by the need of invasive techniques and time required to recover oocytes and ovarian tissue for female fertility preservation, whereas male patients can immediately be planned for banking of several sperm samples. Another aspect of the gender difference is the long-term categorization of procedures for female fertility preservation as “experimental methods.” The label “experimental” was only relatively recently removed (2013) for the cryopreservation of oocytes by the American Society of Reproductive Medicine (23) but it still remains with regard to OTC for fertility preservation, which is obviously an additional barrier for many female patients. The data collected here from academic reproductive medicine clinics in Nordic countries is in line with previous research findings of gender differences in access to and performance of fertility preservation in Sweden (26,27), irrespective of the fact that the procedures for fertility preservation are reimbursed for all patients.

The performance of OTC for women aimed at fertility preservation in Gothenburg, Sweden, as early as 1995, is particularly noteworthy, because these patients were

perhaps the first ones in Europe to undergo this procedure. To the best of our knowledge, centers that have reported early experiences with OTC within fertility preservation programs include the Catholic University of Louvain in Belgium, which was granted approval for OTC in 1995 (28); the Groupe Hospitalier Pitié-Salpêtrière in Paris, which initiated OTC for adult women in 1998 and for prepubertal girls in 2000 (29); and the Free University of Brussels, which initiated OTC in 1999 (30). Similar to the group of Sahlgrenska Hospital in Gothenburg, all these three centers also initiated their OTC programs using the slow-freezing protocol developed by Gosden *et al.* (13).

In conclusion, fertility preservation is gaining ground as an integral and important part of cancer treatment in most Nordic hospitals, for both women and men. Denmark and Norway have national centralized programs for OTC and in Sweden and Finland the regional programs together cover the whole population. However, not all patients are counseled before potentially gonadotoxic treatment and national differences are evident. Further investigation is needed to identify causes of gender differences in healthcare provision. The solid foundation of fertility preservation services in the public healthcare system, which provides free-of-charge care for eligible

patients, has paved the way for increased implementation of fertility preservation services during the coming years.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Questionnaire regarding cryopreservation of ovarian tissue for fertility preservation in the Nordic Countries