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Transplantation of cryopreserved ovarian tissue in a series of 285 women: a review of five leading European centers

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The feasibility of freezing and thawing ovarian tissue is nowadays widely documented. However, ovarian tissue transplantation (OTT) is happening at a much slower pace, and clinical experience is somewhat limited. In this review, five European centers present their collective experience of transplanting ovarian tissue in 285 women. The focus is on surgical techniques and OTT outcomes, reproductive outcomes, the impact of chemotherapy before ovarian tissue cryopreservation (OTC), the risk of relapse, and endocrine resumption and longevity of transplanted tissue.

The risk of relapse due to reimplantation of ovarian tissue appears to be very low according to current data. Recovery of endocrine function is seen in almost all women undergoing transplantation of ovarian tissue, and about one in four gives birth to a healthy child. The efficacy of in vitro fertilization in these patients is not very high, however, and needs to be substantially improved. Radiation to the pelvis, especially with relatively high doses, appears to considerably decrease the likelihood of a successful pregnancy and may be contraindicated. Our results demonstrate that chemotherapy before OTC does not impair the chances of success, depending, of course, on the total dose and type of chemotherapy administered.

At this early stage of development of OTT for restoration of fertility, the results are encouraging and demonstrate clear potential. However, the method is far from being fully developed and requires continued research efforts to optimize our approach. (Fertil Steril® 2021;115:1102–15. ©2021 by American Society for Reproductive Medicine.)

Key Words: Ovarian tissue transplantation, fertility preservation, ovarian tissue

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In recent years, demand for fertility preservation for oncologic and nononcologic indications, as well as personal reasons, has risen dramatically and will prove a major challenge in the coming years (1). At present, embryo cryopreservation, mature oocyte cryopreservation after ovarian stimulation, and ovarian tissue cryopreservation (OTC) are all fertility preservation approaches endorsed by the American Society for Reproductive Medicine (2, 3).

Of all available means of fertility preservation, oocyte cryopreservation by vitrification (very rapid freezing) provides the highest yield in terms of subsequent pregnancies (4–6), both for women with benign diseases or seeking fertility preservation for personal reasons, and those affected by cancer (if treatment can be postponed) (Fig. 1). This subject is discussed by Cobo et al. in the current issue. OTC is specifically indicated for adolescents and women whose cancer treatment cannot be delayed (7–11) (Fig. 1).

Fertility preservation remains a challenge, particularly in cases of hematologic cancers (Hodgkin's lymphoma, non-Hodgkin's lymphoma, and leukemia) and breast cancer. These cancers are the most common indications for fertility preservation, because chemotherapy (especially with alkylating agents), radiotherapy, surgery, or a combination thereof can induce premature ovarian insufficiency (POI) in certain circumstances (7, 9, 12–15).

Cyclophosphamide is the alkylating agent that causes most damage to resting follicles, including oocytes and granulosa cells, and its effects are dose dependent (16–18). Pelvic radiotherapy is known to induce POI, since exposure to 5–10 Gy is toxic to oocytes (19, 20). Selection criteria need to be established, the most important being age below 35 years (when the ovarian stockpile is still relatively high), a realistic chance of surviving for 5 years, and at least a 50% risk of POI (1, 12, 21). Fertility preservation should also be available to women with certain benign conditions that could lead to POI and those who wish to postpone pregnancy for various personal reasons, the biggest threat to their fertility being age.

Although 7,800 women have undergone OTC in these five European centers, clinical experience with ovarian tissue transplantation (OTT) is still rather limited. The aim of this review was to pool OTT experience from five renowned European centers and analyze important outcomes of the technique in depth. This review focuses specifically on the indications for and results of OTT.

METHODS

A literature search was conducted on the PubMed database for peer-reviewed publications in English using the following key words: "ovarian tissue," "transplantation," "autotransplantation," and "fertility restoration." The aim was to scrutinize case series reported by European clinical centers or groups on OTT outcomes up to October 2020. The selection criteria required case series with more than 20 subjects undergoing transplantation, yielding five centers in Europe: the FertiPROTEKT network covering Germany, Switzerland, and Austria (22–24); the Danish cohort (25); the Spanish cohort (26); the French cohort (27); and the Belgian cohort, with data from Cliniques Universitaires Saint-Luc (1). Each group was then invited to update their data in terms of patient characteristics, OTC and OTT procedures, and fertility outcomes for the present article.

RESULTS

Patient Characteristics upon OTT

Since publication of the first-ever live birth resulting from OTT (28), a number of teams have published their OTT results in the form of either case reports or patient series. So far, more than 200 live births have been reported in the literature (29). However, because the exact number of transplantations remains unknown, it is crucial to analyze data from experienced centers with a known number of transplantations and pregnancies, taking into account all available data.

The current multicenter series includes patients who underwent OTT by one of five teams: Andersen's team from Denmark (62 patients), Diaz's team from Spain (53 patients), Donnez and Dolmans' team from Belgium (29 patients), Poirot's team from France (53 patients), and the FertiPROTEKT network group from Germany, Switzerland, and Austria (88 patients). The total number of subjects enrolled in this series was 285.

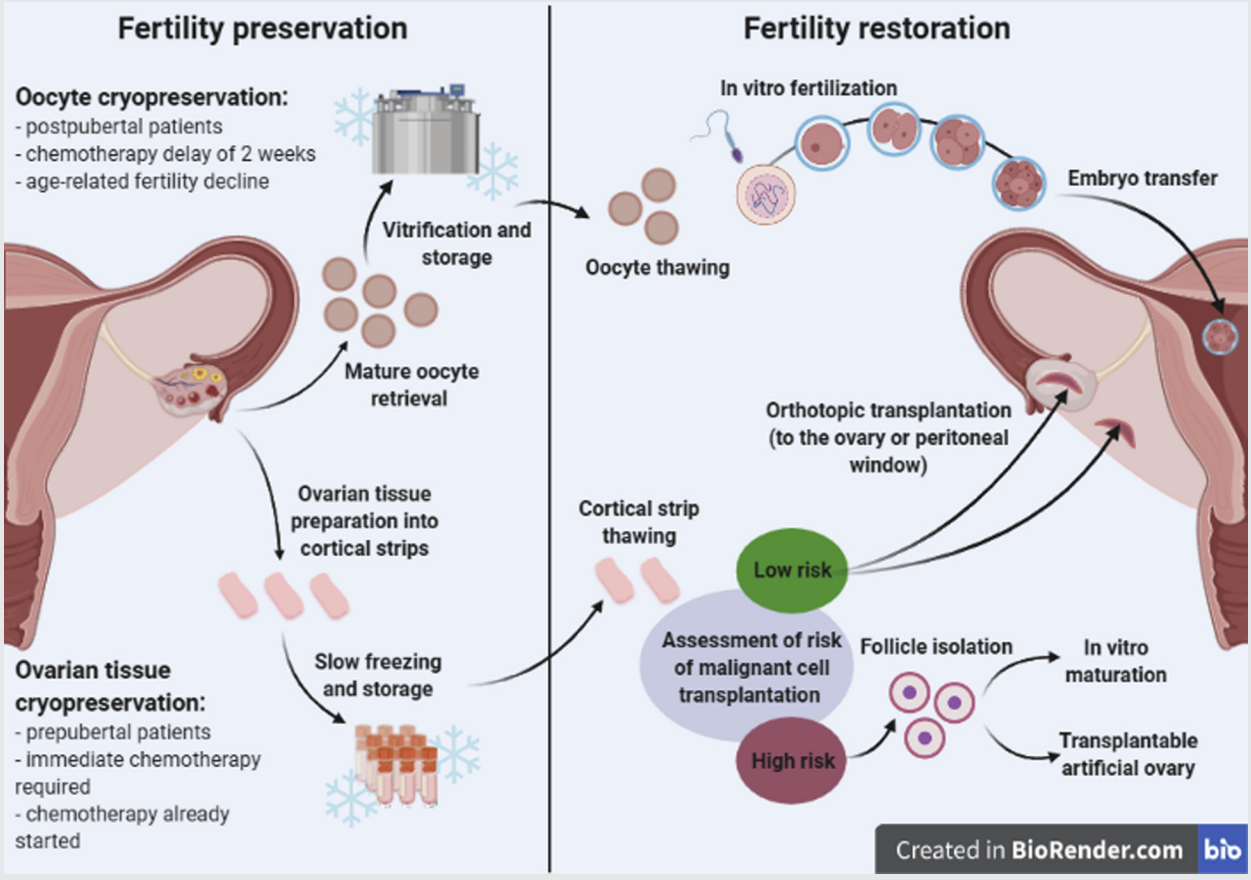
Among patients in whom their tissue was reimplanted, 88.7% had a malignant disease and 11.3% had a nonmalignant disease. Hematologic cancers accounted for 37.2% of patients with malignant diseases, including Hodgkin's lymphoma (24.6%), non-Hodgkin's lymphoma (11.2%), and leukemia (1.4%), followed by breast cancer (33.3%). Other cancerous diseases included the following, in decreasing order of frequency: cancers of the digestive tract, cervical cancer, borderline ovarian tumors, Ewing sarcoma, and others such as melanoma. The distribution of malignant diseases was not identical in the five centers. Breast cancer was the most frequently observed in three centers (Andersen's team, Diaz's team, and the cohort of the FertiPROTEKT network), and Hodgkin's lymphoma was the most frequently observed in the other two centers (Donnez and Dolmans' team and Poirot's team). Among nonmalignant diseases, hemoglobinopathies (9/285; 3.1%), autoimmune diseases (9/285; 3.1%), and aplastic anemia (5/285; 1.7%) were the most frequently observed.

In this cohort of 285 patients, the mean age was 29.3 ± 6.2 years (range, 9–44) at the time of OTC and 34.6 ± 5.5 years at the time of the first OTT. Fifty-nine patients underwent a second OTT and 7 patients a third. From the available data, we know that 81.2% (212/261) of subjects were in POI, and the remaining 18.8% (49/261) were still having irregular menses and showed evidence of infertility. It is important to note that these patients presented with failure of in vitro fertilization (IVF) treatment, and it was decided to boost their ovarian reserve by OTT.

Surgical Techniques and Live Birth Rates

Ovarian tissue can be transplanted to orthotopic sites, namely, the pelvic cavity (back to the medulla of the ovary or a specially created peritoneal pocket) (Fig. 2), or heterotopic sites, namely, outside the pelvic cavity (the forearm or

FIGURE 1



Patients of postpubertal age, patients in whom chemotherapy can be postponed for approximately 2 weeks, or those in a fertility preservation program for age-related fertility decline undergo mature oocyte retrieval after controlled ovarian stimulation. Oocytes are cryopreserved by vitrification. After thawing, they can be fertilized in vitro to obtain embryos for transfer to the uterine cavity. Patients of prepubertal age, patients requiring immediate chemotherapy, or those who have already started chemotherapy undergo ovarian tissue cryopreservation. Ovarian tissue is removed in the form of multiple biopsy specimens, cut into cortical strips, and cryopreserved by slow freezing. After thawing, if there is no risk of malignant cell transmission, the tissue can be grafted to the ovarian medulla (if at least 1 ovary still remains) or reimplanted inside a specially created peritoneal window. If there is a risk of malignant cell transmission, ovarian follicles can be isolated and either grown in vitro to obtain mature eggs or used to create a transplantable artificial ovary. Figure created in BioRender.com.

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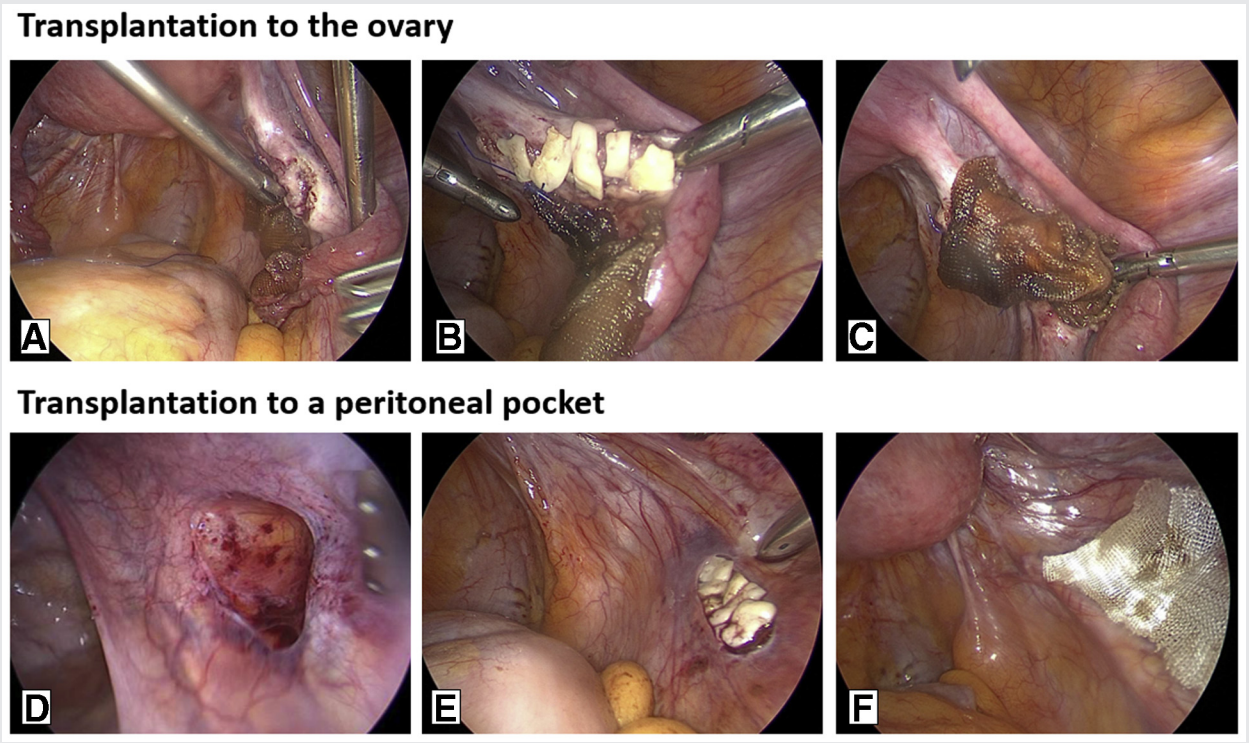
abdominal wall muscle) (30). Of all existing surgical techniques for OTT, orthotopic reimplantation has proved to be the most effective in terms of both resumption of endocrine function and restoration of fertility. It is carried out by laparoscopy or minilaparotomy.

In our series of 285 patients, 277 (97.5%) underwent orthotopic OTT, 3 patients underwent both orthotopic and heterotopic transplantation, and 5 patients underwent only heterotopic (subcutaneous [abdominal wall or forearm]) transplantation. Among those undergoing orthotopic transplantation, 16.7% were grafted to the exposed medulla of decorticated ovaries, 62.7% to a newly created peritoneal window, and 20.4% to both the ovarian medulla and the peritoneal window.

Two important remarks need to be made. First, successful OTT approaches should adhere to fundamental microsurgical principles, ie, finding a well-vascularized transplantation site and a way to affix the ovarian tissue. Second, caution should be exercised when selecting the amount of tissue for transplantation, anticipating the potential need for further reimplantation in the same patient. It is recommended that only part of the cryopreserved tissue be grafted, at least when a large amount was initially cryopreserved (as in the case of ovariectomy or bilateral biopsies). This option should take into account the size of the ovarian reserve at the time of OTC.

Eight women gave birth to more than one infant. Analyzing the number of infants born from each surgical technique, the rates were 30.5% with OTT to the ovary, 34.8% with OTT to the peritoneum, and 34% with the combined technique, indicating that the different sites are equivalent with respect to reproductive efficacy. No pregnancies were obtained from heterotopic grafts, however, showing that this approach is much less effective for reproductive performance.

FIGURE 2



Ovarian tissue transplantation to the ovary: (**A**) Image of a decorticated ovary showing the medulla and its vascular network. (**B**) Ovarian cortical pieces placed in the denuded ovary without stitches and (**C**) subsequently covered and fixed with Interceed or fibrin glue. Ovarian tissue transplantation to a peritoneal window: (**D**) Creation of a peritoneal window using scissors. (**E**) Ovarian cortical fragments positioned with the cortex surface facing the abdominal cavity and (**F**) covered and fixed with Interceed or fibrin glue.

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Live Birth Rates: Spontaneous Conception vs. IVF

Of 285 women who had their frozen-thawed ovarian tissue transplanted in the hope of conceiving, 26% became pregnant and gave birth to one or two infants out of a total of 95 healthy newborns (Table 1). The live birth rate was 30.6% (15/49) in women who had persistent irregular menses while undergoing OTT and 25.4% (54/212) in women who had amenorrhea before OTT. No significant difference in live birth rates was observed between the groups.

The conception rate in women conceiving naturally (40%) was comparable to that achieved with IVF treatment (36%) (Table 1). The live birth rate, although not significantly different, was higher in women conceiving naturally (30%) than in those undergoing IVF (21%). Miscarriage rates, on the other hand, were higher (18%) in subjects receiving IVF treatment than in those conceiving naturally (10%). Furthermore, women who became pregnant were significantly younger at OTC than those who did not become pregnant (mean age, 26.9 ± 0.7 vs. 29.8 ± 0.4 years) (Table 1).

A more detailed picture of IVF outcomes is provided in Tables 2 and 3. It is noteworthy that only 50% of IVF patients actually underwent embryo transfer, despite repeated attempts at ovarian stimulation in many cases. However, a relatively large proportion of those who did undergo embryo transfer conceived (72%) and gave birth (43%), even if the rate of miscarriage was high (37%) (Table 3). Nevertheless, IVF outcomes in terms of obtained embryos were not nearly as good as results achieved with standard IVF treatment (Table 3). Only one in three oocytes developed into an embryo, and quite a large number of patients were unable to produce a single oocyte, despite numerous attempts at ovarian stimulation, partly reflecting an observed empty follicle rate of 31%. The small number of oocytes retrieved during IVF clearly demonstrates that women undergoing transplantation with frozen-thawed ovarian tissue should be considered poor responders right from the start. However, this should not come as a surprise on the basis of simple mathematical calculations. Indeed, if one ovary is excised and frozen, 50% of the ovarian reserve is effectively stored. Upon transplantation to the now-postmenopausal woman, perhaps one third of the stored tissue is transplanted (around 16%–17% of the ovarian reserve before freezing). Moreover, even though cryopreservation is documented to result in follicle survival rates of at least 80% (31), the association between freezing and transplantation may yield overall survival rates of only 30%–50% of follicles, which at this stage represents no more than 5%–8% of the ovarian stockpile at cryopreservation. If a woman is already in her mid-to-late thirties at freezing, only about 5%–10% of the follicles that she had at birth

TABLE 1

Pregnancy outcomes and age of women undergoing frozen-thawed OTT followed by natural conception or IVF.

Method of conception	Women undergoing OTT	Women wishing to conceive	Women who conceived[a]	Women who gave birth	Miscarriages	No. of children[b]	Age (y) at OTC of women who gave birth—mean ± SEM (range)	Age (y) at OTC of women who did not give birth—mean ± SEM (range)
Women conceiving naturally	176	167 (100%)	67 (40%)	52 (30%)	18 (10%)	67	27.6 ± 0.8[c] (17–36)	29.7 ± 0.6[c] (10–44)
Women undergoing IVF	109	109 (100%)	39 (36%)	23 (21%)	20 (18%)	28	25.1 ± 1.2[d] (9–33)	29.9 ± 0.6[d] (17–39)
Total	285	276 (100%)	106 (38%)	75 (26%)	38 (13%)	95	26.9 ± 0.7[e] (9–36)	29.8 ± 0.4[e] (10–44)

IVF = in vitro fertilization; OTC = ovarian tissue cryopreservation; OTT = ovarian tissue transplantation; SEM = standard error of the mean.

[a] Some women may have become pregnant and suffered a miscarriage before a successful subsequent pregnancy, explaining why "women who gave birth" plus "miscarriages" does not add up to "women who conceived."

[b] Some women became pregnant more than once after OTT or had a twin pregnancy.

[c] $P = .046$.

[d] $P = .0002$.

[e] $P = .0005$ (Student's *t*-test).

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remain (32). This may leave her with a very limited number of surviving follicles and therefore a reduced chance of conceiving. These women also face obstetric challenges that increase the risk of miscarriage, such as radiation to the pelvis, uterine surgery, or surgery to the pelvis causing adhesions or unfavorable conditions for OTT.

Despite a considerably diminished ovarian reserve, it is encouraging to observe a spontaneous pregnancy rate of 30%, with only a modest miscarriage rate. This suggests that the tissue is still capable of producing healthy follicles, albeit at low rates, and often in an environment of deficient or even undetectable concentrations of antimüllerian hormone (33–35).

There are several possible explanations for the poor IVF results seen so far, which need to be addressed to optimize outcomes:

Given the low ovarian reserve, only a limited number of follicles are available for recruitment in subjects undergoing grafting. This implies that the normal follicle selection process is not in place, which may allow less viable follicles to survive, particularly with ovarian stimulation, leading to growth and development of follicles that are unable to support a viable oocyte and oocytes that cannot sustain fertilization. This is corroborated by an empty follicle rate of 31% in the current data and is in accordance with the existing literature (34–37).

The miscarriage rate in patients undergoing IVF is especially high, which may reflect the low ovarian reserve, as discussed above, or the fact that the corpus luteum is unable to provide sufficient progesterone to secure pregnancy, particularly when only a few follicles develop, as has been observed in patients undergoing grafting with controlled ovarian stimulation. Therefore, exogenous progesterone administered to IVF patients through different routes could potentially increase progesterone concentrations to sustain the pregnancy.

The current study found that women in the IVF group who achieved childbirth were almost 5 years younger at the time of OTC than those who did not. It was also noteworthy that the upper age limit for freezing for a woman to have a child in the IVF group was 33 years. It is therefore crucial to recognize that the age of the patient at freezing is of utmost importance and that reproductive outcomes worsen with advancing years (25, 38, 39).

Although the suggestion is unproved and debatable, in cases of failure of IVF, outcomes may potentially be improved by transplanting more tissue to increase the ovarian reserve, particularly in reproductive-age women.

At this early stage in the development of a brand-new technique for restoration of fertility in subjects with a relatively low ovarian reserve as the starting point, the results are largely encouraging and reveal clear fertility potential. It is nevertheless important to stress that the highest conception and live birth rates were observed in patients who had conceived spontaneously, suggesting that IVF should not be initiated immediately when there is a chance of spontaneous pregnancy.

Reimplanted tissue is still functioning in many women in the current study, and more pregnancies are likely to follow. However, these are early days, and much more research and

TABLE 2

Women undergoing frozen-thawed OTT followed by IVF.

Women	Ovarian stimulation and IVF	OPU[a]	ET	Women who conceived	Women who gave birth	Miscarriages
All women	109	85 (78%)	54 (50%)	39 (36%)	23 (21%)	20 (18%)
Women undergoing OPU	–	100%	64%	46%	27%	24%
Women undergoing ET	–	–	100%	72%	43%	37%

ET = embryo transfer; IVF = in vitro fertilization; OPU = oocyte pick-up; OTT = ovarian tissue transplantation.
[a] The empty follicle rate was available for 53 women, yielding a 31% rate of follicles without an oocyte (range, 0–100).

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development is required to optimize methods and define age limits and the most appropriate stimulation regimens.

OTT After Pelvic Irradiation

If the pelvis needs to be irradiated, OTC and OTT require specific considerations. In our cohort of 285 patients, 36 women (12.6%) received some kind of pelvic irradiation before OTT Among these 36 women, 9 had been diagnosed with anal cancer, 8 with colorectal cancer, 6 with cervical cancer, 6 with Hodgkin's lymphoma, 2 with non-Hodgkin's lymphoma, 1 with leukemia, 1 with Ewing sarcoma, 1 with sickle cell disease, 1 with vulvar cancer, and 1 with uterine cancer. In this group of 36 women, 9 pregnancies and 7 live births (19%) were reported, with differences depending on the disease in question.

In women with anal or cervical cancer (n = 15) requiring a high radiation dose, no live births were obtained. One live birth was obtained in patients with colorectal cancer (n = 8) or Ewing sarcoma (n = 1), and 5 live births were reported in patients with systemic diseases (n = 10), such as lymphoma, leukemia, and sickle cell disease, mainly requiring total body irradiation (TBI) with a lower pelvic irradiation dose.

Clearly, live birth rates after OTT depend on the delivered dose of pelvic irradiation. Anal and cervical cancer require high doses of pelvic irradiation (0% births in our study). In colorectal cancer, the irradiation dose was reduced (12.5% of patients giving birth), and it was even lower in cases of TBI (50% of patients giving birth). The main reasons for low live birth rates after high-dose radiotherapy are probably linked to poor vascularization of the transplant due to fibrosis of pelvic tissue, including the peritoneum, and irradiation of the uterus.

Pregnancies following uterine irradiation are at risk for complications such as preterm birth and low birth weight (LBW) (40). Salooja et al. (41) compared births to women who underwent stem cell transplantation with and without TBI. The proportion of infants with birth weight <2,500 g was about 30% among those whose mothers underwent TBI vs. 10% among those whose mothers did not have TBI. Evaluating childhood cancer survivors (n = 340), Chiarelli et al. (42) found an increased risk of preterm birth and LBW infants (odds ratio [OR] 3.49, 95% confidence interval [CI] 1.26–9.72) with doses of >2.5 Gy to the pelvis and/or abdomen. Signorello et al. (43) investigated the offspring of 1,264 cancer survivors vs. a group of 601 female siblings and found increased risks of preterm birth (OR 3.5, 95% CI 1.5–8) and LBW (OR 6.8, 95% CI 2.1–22.2) if the uterus was irradiated with >5 Gy.

Data on pregnancies following high-dose pelvic radiotherapy are quite sporadic and are essentially case reports. Hürmüz et al. (44) reported a spontaneous singleton pregnancy after pelvic radiotherapy with 30 Gy without any pregnancy complications. De Menezes et al. (45) described a twin pregnancy after egg donation following radiotherapy to the right pelvis with 36 Gy, with cesarean section in the 35th week of pregnancy due to pre-eclampsia. Bath et al. (46) recorded a complication-free spontaneous singleton pregnancy following radiotherapy to the left pelvis with 55 Gy and the right pelvis with 10 Gy.

Recommendations regarding maximum radiation doses to the uterus were published by Teh et al. (47). Following

TABLE 3

Outcomes of ovarian stimulation and IVF in 109 women undergoing frozen-thawed OTT.

	No. of cycles	No. of oocytes retrieved[a]	No. of embryos (per oocyte retrieved)[b]	No. of ETs (per cycle)[c]
Overall (total)	428	525	169 (32%)	150 (35%)
Per woman (total)—mean ± SEM (range)	3.7 ± 0.3 (0–28)	6.2 ± 0.9 (1–32)	3.1 ± 0.5 (1–24)	2.3 ± 0.3 (1–18)

ET = embryo transfer; IVF = in vitro fertilization; OTT = ovarian tissue transplantation; SEM = standard error of the mean.
[a] With at least one oocyte.
[b] With at least one embryo.
[c] With at least one ET.

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systematic analysis of the clinical consequences of radiotherapy in childhood and adulthood, they came to the following conclusions and guidelines:

Radiotherapy to the adult uterus with TBI (12 Gy) is associated with increased risks of miscarriage, premature birth, and LBW.

If the uterus is irradiated with >25 Gy in childhood, pregnancy is not recommended.

If the uterus is irradiated with >45 Gy in adulthood, pregnancy is contraindicated.

Uterine radiation in childhood was associated with greater adverse effects on uterine reproductive capacity, with evidence of irreversible uterine damage from lower direct radiation doses (>25 Gy) compared with adults (>45 Gy). If the administered irradiation dose allows transplantation, the tissue should be grafted to an area that received the lowest dose. If orthotopic transplantation is impossible, subperitoneal transplantation outside the pelvis can be considered, although it appears to yield very low success rates (48).

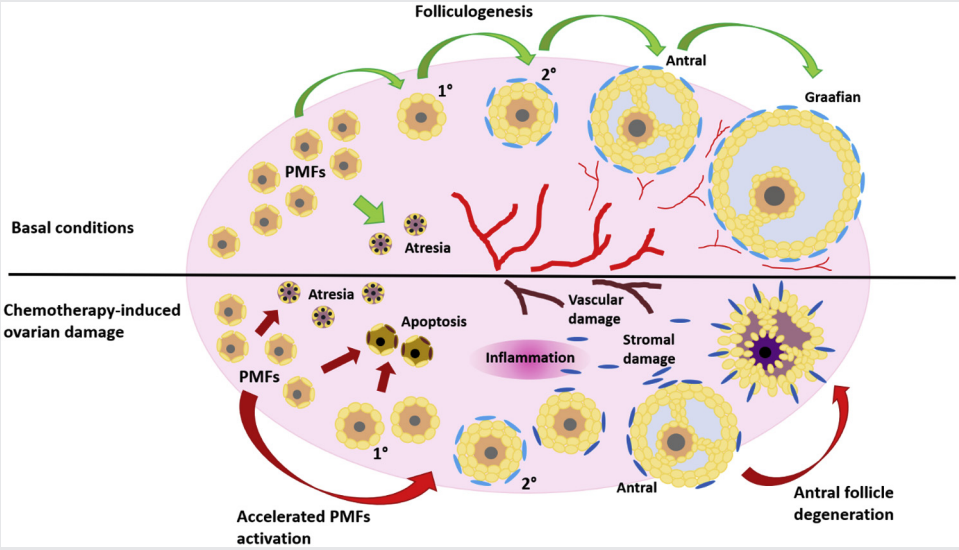
In summary, ovarian tissue can be transplanted after pelvic radiotherapy if the radiation exposure was relatively low, as in the case of TBI. However, because of the poor success rates and obstetric risks associated with diseases requiring high pelvic radiation doses, such as anal, rectal, and cervical cancer, OTC and OTT should be carefully considered before going ahead. Our data have nevertheless demonstrated that ovarian tissue can be transplanted after radiation, but probably only according to specific criteria, taking into account not only the disease, but also the radiation zone and dose. Moreover, before OTT is contemplated, endometrial thickness and function need to be evaluated.

Effect of Chemotherapy Before OTC on the Results of OTT

It has been shown that chemotherapy, in addition to causing quantitative alterations to the pool of follicles in the reserve, can lead to qualitative alterations of follicles and oocytes, as well as vascular damage, with subsequent fibrosis in the ovary (49–53) (Fig. 3). It is therefore usually recommended that OTC be performed before the start of chemotherapy in patients over 15 years of age (21). However, there are some situations in clinical practice, such as acute leukemia, where OTC is not feasible before the start of chemotherapy, and recent recommendations issued by European Society of Human Reproduction and Embryology (ESHRE) indicate that "patients who have already received low gonadotoxic treatment or a previous course of chemotherapy can be offered OTC as a fertility preservation option" (3). Given this paradoxical situation, exploring the results of OTT in patients previously exposed to chemotherapy is vital in order to offer them the best possible treatment.

Overall results for the present cohort. In the present cohort of 285 patients, data on chemotherapy were available for 271 patients. Fifty patients (18.5%) were exposed to chemotherapy before OTC. A great disparity was noted between centers, with prior exposure to chemotherapy ranging from 0% to 66%. In patients who had undergone chemotherapy before

FIGURE 3



Chemotherapy exerts numerous damaging mechanisms on the ovary: loss of primordial follicles by apoptosis, accelerated activation of primordial follicles, enhanced follicular atresia, inflammation in the stroma, and damage to the intraovarian vascularization. PMFs = primordial follicles. Reproduced with permission from (52).

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OTC, the rate of resumption of ovarian function was not significantly different from that in patients who had not undergone chemotherapy before OTC (90% vs. 85.3%; $P = .49$). The rates of pregnancy in subjects grafted with ovarian cortex who were previously exposed to chemotherapy were 50.0% vs. 28.1% in those who had not undergone chemotherapy before OTC ($P = .004$).

Although the median ages of women who had undergone chemotherapy before OTC (26 years; range, 12–35) and those who had not (31 years; range, 9–44) were significantly different ($P < .0001$), logistic regression including age at OTC, prior chemotherapy exposure, and the clinical center as covariates showed that age (OR 0.91, 95% CI 0.87–0.96, $P < .001$) and prior chemotherapy (OR 4.20, 95% CI 1.64–10.73, $P = .003$) were independently associated with the likelihood of conception. Univariate and multivariate analyses of pregnancy outcomes suggest that exposure to chemotherapy before OTC does not alter the results of OTT and is no longer a contraindication to freezing, as demonstrated by previous studies (27, 54–56) (Table 4).

Complementary study on a matched series. Because of the heterogeneity of patients and in order to confirm these results, we performed a matched study. The patients were divided into two groups: group 1, patients exposed to chemotherapy before OTC (chemo+ group); and group 2, patients not exposed to chemotherapy before OTC (chemo– group). The subjects were matched for age and disease. Each patient in the chemo+ group was paired with a chemo– patient of the same age and with the same disease. In one case, two patients in the chemo– group corresponded to one patient in the chemo+ group, and both were kept.

Twenty-nine patients from the chemo+ group were thus matched with 30 patients from the chemo– group. The results confirmed that there was no difference between the two groups in the rate of recovery of ovarian function and that the rates of pregnancy and live births were higher in the chemo+ group. Some explanation for these results was provided in a recent article showing that ovarian tissue from women treated with the ABVD regimen (chemotherapy free of alkylating agents) had a higher density of primordial follicles than tissue from patients with lymphoma who had not received chemotherapy or those treated with OEPA-COPDAC, a chemotherapy regimen containing alkylating agents (57). One hypothesis is that some chemotherapy could possibly prevent follicle activation. Future research into signaling pathways involved in follicle activation, such as PI3K/Akt, may shed light on this subject.

Effect of alkylating agent–containing chemotherapy regimens on OTT outcomes. In the population of women exposed to chemotherapy before OTC, women treated with alkylating agents had a lower rate of pregnancy than women treated with chemotherapy without alkylating agents (32.1% vs. 68.4%, $P = .019$) and a lower rate of giving birth to at least one child (28.6% vs. 68.4%, $P = .016$). This study confirms the harmful effects of alkylating agents on OTT results (55).

In conclusion, to our knowledge, this series is the largest cohort of women exposed to chemotherapy before OTC ever published and offers further evidence that chemotherapy before OTC does not alter the results of OTT and is no longer a contraindication. Fertility preservation guidelines now need to be updated to state that OTC should be the fertility preservation approach of choice when chemotherapy has already begun, because mature oocyte cryopreservation is not effective in this case (58).

Resumption of Endocrine Function and Longevity of Transplanted Ovarian Tissue

Of 258 patients who underwent OTT and had available follow-up data, 204 received a diagnosis of POI, of whom 181 (88.7%, ranging from 77.6% to 97.2 % according to center) had resumption of endocrine function, as shown by resumption of menstruation. The mean ± SD interval between transplantation and first menstruation was 4.5 ± 2.2 months. Although recovery of ovarian function was achieved at 4 to 5 months after grafting in the majority of cases, some

TABLE 4

Main series of OTT patients exposed to chemotherapy before OTC.

Authors and year of publication	History of chemotherapy before OTC (n = number of patients)	% of women giving birth to at least 1 child
Meirow et al. 2016 (54)	No chemotherapy (n = 10)	20%
	Chemotherapy (n = 10)	40%
Poirot et al. 2019 (27)	No chemotherapy (n = 9)	0%
	Chemotherapy (n = 22)	32%
Poirot et al. 2019 (55)	OTC >3 months after the end of chemotherapy (n = 10)	40%
	OTC during chemotherapy (n = 15)	46.7%
	HL treated by different types of chemotherapy before OTC (n = 16)	50%
	HL treated by ABVD before OTC (n = 8)	62.5%
Shapira et al. 2020 (56)	No chemotherapy (n = 36)	38.9%
	Chemotherapy (n = 24)	45.8%
Present study	No chemotherapy (n = 221)	20.4%
	Chemotherapy (n = 50)	44%

ABVD = adriamycine, bleomycine, vinblastine, dacarbazine; HL = Hodgkin's lymphoma; OTC = ovarian tissue cryopreservation; OTT = ovarian tissue transplantation.

Dolmans. Ovarian tissue transplantation outcomes. Fertil Steril 2021.

variability was observed. The average interval for resumption of ovarian function is consistent with the folliculogenesis time frame, that is, the time needed for primordial follicles to develop to the antral stage (59). Quiescent follicles are less sensitive to cryoinjury (60, 61) and therefore constitute the main population in frozen-thawed ovarian tissue. Growing follicles, on the other hand, appear to be more vulnerable not only to freezing and thawing, but also to ischemic injury occurring soon after OTT. Various factors, such as patient age, interpatient variability in the ovarian reserve, and exposure to gonadotoxic chemotherapy before OTC, are critical not only to the success of OTT, ie, endocrine resumption and restoration of fertility, but also to ovarian function lifespan (62).

In the present case series, 59 of 285 patients (20.7%) underwent a second OTT procedure and 7 (2.4%) underwent a third procedure, with a mean interval of 2.5 years between procedures. A second or third procedure was indicated when a woman had poor recovery of ovarian function after grafting.

A subgroup of 45 subjects who underwent transplantation more than 5 years ago and with complete hormonal follow-up information was evaluated to determine ovarian function longevity, irrespective of the number of transplantations per patient (Fig. 4). The results showed a 5-year graft survival rate of 55% and a significant correlation between the duration of ovarian function and age at the time of OTC, regardless of whether chemotherapy was previously administered. These findings are in line with those from the first case series, which reported a mean ovarian graft lifespan of 4 to 5 years (63, 64). Of the three patients with the longest periods of ovarian activity, two underwent grafting three times to reach cumulative periods of ovarian activity of 13.5 and 11 years, and the other patient underwent grafting only once in 2007 at the age of 13 years for induction of puberty (65). Analysis showing a negative correlation between mean graft lifespan and increasing age highlights the crucial importance of the primordial follicle pool at the time of OTT, which is known to be age dependent (32).

The number of primordial follicles that are present in ovarian fragments at the time of transplantation and that survive the grafting procedure appears to be the major determinant of OTT success and graft longevity (66). The ovarian reserve suffers major follicle depletion after OTT, with a follicle loss of 50%–90% shortly after transplantation (67, 68). Moreover, abnormal acceleration in follicle growth has also been observed soon after OTT (69–71), further contributing to ovarian reserve depletion. This follicle "burn-out" appears to be due mainly to abnormal activation of signaling pathways, such as the PI3K/Akt pathway, that are involved in cell proliferation and survival and are responsible for primordial follicle activation in the early post-transplantation period (71–73).

Numerous research groups are developing strategies to mitigate large-scale follicle loss occurring after grafting by enhancing the revascularization process immediately after transplantation in order to reduce hypoxia-related damage to ovarian tissue. Promising results have been reported with the use of adult stem cells with proangiogenic potential (74, 75), proangiogenic growth factors such as fibroblast growth factor (76), and revascularization enhancers such as sphingosine-1-phosphate (77).

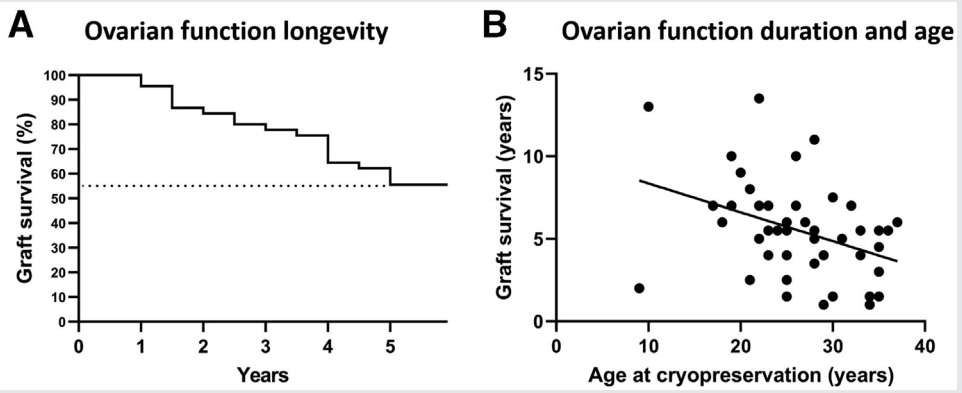
Relapse and Risks: How to Exclude the Threat of Malignant Cell Presence

Safety issues surrounding reimplantation of ovarian tissue from cancer patients have been a cause of concern for many years (78, 79). A number of studies have investigated the risks of reintroducing malignant cells together with frozen-thawed ovarian tissue, which could induce recurrence of the primary tumor (80–82). In 2010, studies by Dolmans and Andersen's group found that the highest risk of spread of tumor cells as a result of OTT was associated with hematologic malignancies, particularly leukemia (83, 84). Although histologic examination failed to detect malignant cells in ovarian tissue from patients with chronic myeloid leukemia or acute lymphoblastic leukemia, disease-specific polymerase chain reaction techniques showed ovarian contamination in 33% of patients with chronic myeloid leukemia and 70% of patients with acute lymphoblastic leukemia (83). Two years later, Greve et al. reported that chemotherapy before OTC could help remove malignant cells from ovarian tissue, even though it could not completely preclude malignant contamination (85). It is reasonable to assume that ovarian tissue from a patient in complete remission is less likely to harbor malignant cells than tissue from a patient in the active disease phase and is less prone (ie, with a much lower risk) to induce recurrence after grafting (85, 86). A correlation between bone marrow status on the day of ovarian biopsy and ovarian contamination was found by Jahnukainen et al. (87) but could not be confirmed by Chevillon et al. (86). In any case, all these studies provide clear arguments in favor of harvesting ovarian tissue while patients are in complete remission, especially since neither graft follicle density nor reproductive performance is significantly affected by chemotherapy administered before OTC (55, 88, 89). The present review provides data in favor of this approach. Indeed, fertility outcomes in patients who had received chemotherapy before cryopreservation were not significantly compromised.

In 2018, the first successful autologous reimplantation of ovarian tissue was described in a survivor of acute myeloid leukemia after tissue harvesting during complete remission, combined with meticulous tissue evaluation before OTT (88). In our series of 285 patients, 2 patients with acute myeloid leukemia underwent transplantation, but no relapse was observed in these subjects, who were generally considered to be at higher risk. Their ovarian tissue was frozen after chemotherapy in both cases (27, 55).

In the present series of patients undergoing OTT, 12 (4.2%) had a relapse. More than half of them were breast cancer patients (n = 7), and the remaining 5 were suffering from Ewing sarcoma, cervical cancer, non-Hodgkin's lymphoma, anal carcinoma, and a central nervous system tumor (Table 5). In these 12 patients, the time from transplantation to relapse ranged from 2 months (in a breast cancer patient who was 44 years of age at OTT) to 10 years (in a patient with a central nervous system tumor who had already given

FIGURE 4



(**A**) Ovarian function longevity in a subgroup of 45 subjects transplanted more than 5 years ago and with complete hormone follow-up over time, calculated using the Kaplan-Meier plot. (**B**) A negative correlation was seen between ovarian function duration and increasing age in the same subgroup of patients ($y=-0.174x + 10.1$; $p=0.009$; $R^2=0.146$).

Dolmans. Ovarian tissue transplantation outcomes. Fertil Steril 2021.

birth to three children from her ovarian graft) (90, 91). Of course, the timing of relapse is totally unpredictable. Even if great care is taken when planning reimplantation (patient in remission), patients may still relapse very soon after. A breast cancer patient who relapsed 6 months after OTT (Table 5) was in week 8 of a spontaneous pregnancy but had to abort in order to undergo chemotherapy (92). One patient with Ewing sarcoma relapsed 4.5 years after OTT (93), but this is known to be an aggressive disease with occasional ovarian involvement (94, 95). Indeed, patients with sarcoma who have ovarian tissue cryobanked have the highest death rate among patients with stored ovarian tissue, estimated to be more than 30% (33% in the Belgian series [96] and 31% in the Danish series [97]). However, even in the presence of aggressive malignancies, OTC should still be proposed to all sarcoma patients, as recent studies show no particular risk of ovarian involvement in ovarian tissue from sarcoma patients (81, 98).

It is crucial to stress that all relapses (n = 12) were dependent on the primary disease and not related to OTT (26, 90, 92, 93, 99–102). All were distant from the grafting site, and most were very close to the location of the primary cancer. A study by Andersen's team came to the same conclusion (25). Worldwide data on OTT reported by that team in 2018 found 9 of 230 women (3.9%) with malignant disease had a relapse after OTT, but none of the cases was thought to be related to their reimplantation procedure (25). The current study confirms this recurrence rate (12/285, 4.2%).

Considering the frequency of breast tumor relapse, a number of points need to be made. Breast cancer is one of the most frequent indications for OTT. Indeed, in this series

TABLE 5

Outcomes of disease relapse after OTT in a series of 285 patients.

Group	Age at OTC (y)	Pathology	Chemotherapy before OTC	Time from OTC to OTT (y)	Duration of follow-up to 01/2020 (y)	Age at OTT (y)	Pregnancy	Timing of relapse
Dolmans	17	CNS tumor	No	7	11.3	24	3 live births	10 y after OTT
Poirot	31.9	NHL	Yes	2.5	5.7	34.4	0	7 mo after OTT
Poirot	36.5	Breast cancer	No	7.8	2.2	44.3	0	2 mo after OTT
Diaz	28	Breast cancer	No	7.97	5.54	35	0	8 mo after OTT
Diaz	37	Breast cancer	No	2	5.52	39	0	1 y after OTT
Andersen	9	Ewing sarcoma	No	4.4	5.2	9	0	4.5 y after OTT
Andersen	33	Breast cancer	No	5.2	6.9	33	1	Relapse in the left breast
Andersen	30	Cervical cancer	No	1.5	12.7	30	0	Relapse in the uterus
FertiPROTEKT	28	Breast cancer	No	5.0	6.0	33	0	NA
FertiPROTEKT	28	Breast cancer	No	6.0	NA	34	0	NA
FertiPROTEKT	32	Anal carcinoma	No	2.0	3.0	34	0	NA
FertiPROTEKT	36	Breast cancer	No	3.0	3.0	39	1 live birth	NA

CNS = central nervous system; NA = not available; NHL = non-Hodgkin's lymphoma; OTC = ovarian tissue cryopreservation; OTT = ovarian tissue transplantation.

Dolmans. Ovarian tissue transplantation outcomes. Fertil Steril 2021.

of 285 OTT patients, 96 had breast cancer and 7 of them relapsed (7%). Breast cancer is a disease with a known risk of relapse for many years (103). It is also known that young age at breast cancer diagnosis is associated with an increased risk of recurrence (104), and patients undergoing OTC are typically young, almost always under 40 years of age at the time of OTC. The prevalence of relapse in these patients (7%) is actually similar to that observed in the literature generally for women with breast cancer aged under 40 years. The rates of local recurrence range from 10% after breast-conserving surgery (105) to 4%–8.7% at 10 years (103). When undertaking OTT in these patients, we should always remember that they remain cancer patients in remission, and that gynecologic, endocrine, and reproductive follow-up should take this fully into account, according to oncologic guidelines published by international societies (3, 106).

CONCLUSIONS

To our knowledge, this is the largest series ever published analyzing individual patient data from five leading European centers. It shows the diversity of diseases in patients undergoing OTT, with cancer clearly dominating. Since efficacy and safety have been demonstrated in the present review, OTC followed by OTT should now enter into clinical routine. Recovery of endocrine function was achieved in almost all women undergoing transplantation of ovarian tissue, and despite low numbers of surviving follicles, ovarian tissue is able to support endocrine function for several years. Many women experienced pregnancy resulting in the birth of healthy offspring, with around one in four giving birth to a healthy child in our large series. Compared with natural conception, the efficacy of IVF in these patients was not very high and needs to be substantially improved.

Our study showed that high doses of radiation to the pelvis reduce the likelihood of a successful pregnancy and may constitute a contraindication. Very interestingly, however, our results also demonstrate that chemotherapy before OTC does not impair the chances of success, depending on the type of drugs used and the total dose administered. We would therefore like to stress some important points concerning patient selection that emerged from the present review. Indeed, although some teams refuse to cryopreserve ovarian tissue if chemotherapy has already started, our series proved that women undergoing, or having undergone, several regimens of chemotherapy can still benefit from OTC, with good results in terms of live births after OTT. Our study also emphasizes that in cases of leukemia, ovarian tissue should be harvested while the patient is in complete remission, especially because neither graft follicle density nor reproductive performance is affected by chemotherapy administered before OTC.

So far, there have been no cases of relapse related to the grafting of ovarian tissue, and the risk of reintroducing malignant cells is very low. The technique is therefore safe and does not pose a health threat with the current indications. The findings of this study should help to modify selection criteria and develop guidelines aiming to promote more efficient and uniform application of OTT for all those who need it.

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