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ORIGINAL ARTICLE *Infertility*

Determinants of transplantation success with cryopreserved ovarian tissue: data from 196 women of the FertiPROTEKT network

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STUDY QUESTION: What are the pregnancy and live birth rates for ovarian tissue transplantation and which factors are associated with the success rate?

SUMMARY ANSWER: Pregnancy and live birth rates per transplanted woman are 32.7% and 26.5% and success rate is associated with female age and first versus repeated transplantation.

WHAT IS KNOWN ALREADY: Live birth rates after ovarian tissue transplantations have been reported to be between around 24% and 41% per patient. Success rates seem to be negatively associated with increasing female age at the time of tissue cryopreservation and with pelvic radiation. Success rates are apparently not reduced after overnight transportation of ovarian tissue before freezing.

STUDY DESIGN, SIZE, DURATION: Registry analysis of 244 transplantations in 196 women, performed by 26 FertiPROTEKT network centres from 2007 to 2019 with follow-up till December 2020.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Orthotopic ovarian tissue transplantations were performed in 196 women, 191 with previous malignant and 5 with previous non-malignant diseases. Size of transplanting centres varied between 1 and 100 transplantations per centre (median: 2). Factors possibly associated with success rate such as female age, first and repeated transplantation, experience of the transplanting centre and overnight transportation of the ovarian tissue before freezing were analysed.

MAIN RESULTS AND THE ROLE OF CHANCE: Average age of all 196 transplanted women was 31.3 years (SD 5.2; range 17–44) at the time of cryopreservation of tissue and 35.9 years (SD 4.8; range 23–47) at the time of transplantation. Pregnancy rate was 30.6% (95% CI, 24.2–37.6%) per first transplantation and 32.7% (95% CI, 26.1–39.7%) per patient. Pregnancy rate was higher after first transplantation (30.6% (95% CI, 24.2–37.6%)) compared to second and subsequent transplantations (11.8% (95% CI, 3.3–27.5%)). Live birth rate per first transplantation was 25.0% (95% CI, 19.1–31.7%) and per patient 26.5% (95% CI, 20.5–33.3%). Success rate decreased with increasing age at the time of ovarian tissue freezing. Live birth rate was 28.2% (95% CI, 20.9–36.3%) in women <35 years and 16.7% (95% CI, 7.9–29.3%) in women >35 years. Pregnancy rates after first transplantation were higher in centres who had performed ≥10 transplantations (35.1%) compared to centres with <10 transplantation (25.4%) ($P = 0.12$). Corresponding live birth rates were 27.0% and 18.6%.

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Success rates were not different in women with and without overnight transportation of tissue before cryopreservation.

LIMITATIONS, REASONS FOR CAUTION: The data were drawn from a registry analysis. Data such as ovarian reserve and premature ovarian insufficiency were not available for all women. Data might be influenced by different follow-up policies of the centres.

WIDER IMPLICATIONS OF THE FINDINGS: The study reveals the high potential of ovarian tissue freezing and transplantation, but only if freezing is performed in younger women. The study suggests focus should be placed on the first and not on repeated transplantations. It also opens the discussion of whether transplantation should rather be performed by experienced centres.

STUDY FUNDING/COMPETING INTEREST(S): No funding. No competing interests.

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Key words: fertility preservation / ovarian tissue / transplantation / pregnancy rate / live birth rate / cancer / FertiPROTEKT

Introduction

The first successful restoration of ovarian endocrine function was achieved in 2000 and the first embryo development in 2004 by transplantation of cryopreserved autologous ovarian tissue (Oktay and Karlikaya, 2000; Oktay *et al.*, 2001, 2004). Shortly thereafter, the first live births after ovarian tissue transplantation were reported (Donnez *et al.*, 2004; Meirow *et al.*, 2005; Marin *et al.*, 2020). Since then, cryopreservation of ovarian tissue has become an increasingly widely used technique for preserving fertility before cytotoxic treatments. More than 200 live births have been reported worldwide (Dolmans *et al.*, 2021). The procedure of ovarian tissue freezing and transplantation has also been incorporated into numerous recommendations and guidelines as an established method, but it still needs improvement (Loren *et al.*, 2013; AWMF, 2017; Beckmann *et al.*, 2018; Oktay *et al.*, 2018; von Wolff *et al.*, 2018; Practice Committee of the American Society for Reproductive Medicine, 2019; Suzuki, 2019; ESHRE Guideline Group on Female Fertility Preservation, 2020; Harada *et al.*, 2022). Data about the success rate, the transplantation technique and the factors associated with transplantation success are still limited. These limitations render it difficult to define reliable indications for ovarian tissue freezing in cases where freezing of oocytes is also a feasible option (von Wolff and Liebenthron, 2020).

The largest study so far, including ovarian tissue transplantations performed in 285 women by 5 large centres or networks in Europe (Dolmans *et al.*, 2021), revealed a live birth rate per woman of 26%. The study confirmed a negative association of the success rate with increasing female age at the time of tissue freezing, which has already been published by others (van der Ven *et al.* 2016). Even though this study is the largest published worldwide, its heterogeneity regarding tissue freezing and transplantation techniques did not allow an analysis of factors such as transportation of tissue before freezing, first and repeated transplantation and the impact of the experience of the transplanting centre.

The feasibility of tissue transportation at around 4°C for 5–24 h had been evaluated previously by Jensen *et al.* (2015) and Liebenthron *et al.* (2019) in smaller cohorts. Previous data on the success rates of first versus repeated transplantation are not conclusive (Jensen *et al.* 2015; van der Ven *et al.*, 2016) and the impact of the experience of the transplanting centre on the success rate has yet not been studied.

The *Ferti*PROTEKT network has frozen ovarian tissue in around 5000 women and performed 244 ovarian tissue transplantations in 196 women until the end of 2019 (Germeyer *et al.*, 2021).

Freezing and transplantation are performed similarly by all centres within the *Ferti*PROTEKT network due to recommendations provided by the network (von Wolff and Nawroth, 2020). However, transplantations are performed by a large number of centres with very different experience.

The aim of the present study was therefore to update the outcome of ovarian tissue transplantations regarding pregnancy and live birth rates performed in the *Ferti*PROTEKT network which reflect the situation in most countries where many centres are involved in transplantation. The study also aimed to define factors associated with pregnancy and live birth rates such as female age, first and repeated transplantation, experience of the transplanting centre and overnight transportation of the tissue before freezing.

Materials and methods

The FertiPROTEKT data registry

FertiPROTEKT (www.fertiprotekt.com) is a network founded in 2006 as an association of 158 university and non-university reproductive medicine centres in Germany (n = 137), Austria (n = 13) and Switzerland (n = 8) (September 2022). It runs a registry, data from which is published on an annual basis in a national and international registry report of the German IVF registry, DIR (https://www.deutsches-ivf-register.de/).

The FertiPROTEKT registry collects data from women who have undergone ovarian tissue transplantation. Women presenting to individual centres who did not receive ovarian tissue transplantation or who underwent ART treatment prior to transplantation have not yet been included in the registry, nor have women with a successful pregnancy without ovarian tissue transplantation after ovarian tissue cryopreservation.

From 2007 until the end of 2020, 281 ovarian tissue transplantations were registered and in 84% of cases, only 50% of one ovary was removed (Germeyer *et al.*, 2021).

The tissue is stored by 26 centres, some of which run large tissue banks which receive the tissue from surrounding centres, partially transported overnight at 4°C (Liebenthron *et al.*, 2019).

Data analysis, characteristics of patients and transplanting centres

From 2007 up to the end of 2019, a total of 244 transplantations had been carried out in 196 women. The transplantations were evaluated until the end of 2020 to allow a follow-up of at least 12 months after transplantation. Of those 196 women, the first 88 had already been

analysed and published (van der Ven *et al.*, 2016; Liebenthron *et al.*, 2019; Lotz *et al.*, 2019; Dolmans *et al.*, 2021; Khattak *et al.*, 2022).

The centres with ≥10 performed transplantations were Erlangen (n = 100), Heidelberg (n = 23), Neuss (n = 19), Bonn (n = 19), Düsseldorf (n = 14) and Tübingen (n = 10), all in Germany.

Collection, transportation and freezing of tissue were performed according to the recommendations provided and published by the network *Ferti*PROTEKT (von Wolff and Nawroth, 2020).

Removal, transportation and freezing of the tissue

Ovarian tissue was collected by laparoscopy in most cases, a laparotomy was only performed if required in the context of the underlying oncological diseases. The tissue was removed without heat coagulation to avoid damaging ovarian tissue. After collection, the tissue was immediately transferred to an infertility centre close by or was transported overnight at around 4°C (n = 98, 50.0%) to one of the centralized cryobanks of the network, where it was frozen and stored in liquid nitrogen.

Tissue was either stored in one of the centralized cryobanks (Bonn, Erlangen and Düsseldorf, n = 141) or in smaller individual centres (n = 55).

Collection of the tissue, transportation and freezing are described in detail by van der Ven *et al.* (2016), Beckmann *et al.* (2019), von Wolff and Nawroth (2020) and Liebenthron and Montag (2021). Transportation of the tissue before freezing is described by Liebenthron *et al.* (2019).

Transplantation technique

Tissue was transplanted in women with premature ovarian insufficiency and in women who did not become pregnant spontaneously and who asked for transplantation to increase their pregnancy chances. The diagnosis of premature ovarian failure was made by each centre according to the definition in the current guidelines (Webber *et al.*, 2016). Hormone levels were not reported to the registry. Each centre made its own decision as to which patient was eligible for transplantation.

Time between tissue freezing and transplantation was 4.6 years (SD 2.7 years; range 1–16 years). The transplantations were carried out according to recommendations provided by *Ferti*PROTEKT (von Wolff and Nawroth, 2020) and are described elsewhere (van der Ven *et al.*, 2016; Beckmann *et al.*, 2019; Liebenthron and Montag, 2021). There were no appreciable differences between centres in surgical techniques.

In brief, after a short incubation period at room temperature, cryotubes were transferred to a 37°C warm water bath before transplantation. The tissue pieces underwent a dehydration and rehydration process with sucrose to remove antifreeze agent and to restore the cell volume. The patency of the uterine tubes was checked, and the tissue was transplanted orthotopically into a peritoneal pocket (87.5%) and/or into one of the ovaries on the side where the tubes had been shown to be patent (4.5%) or to both locations (8.1%).

For transplantation into a peritoneal pocket, the peritoneum was opened in the ovarian fossa. The tissue pieces were introduced into the pocket with the ovarian surface facing the abdominal cavity. The peritoneum was closed with absorbable sutures when necessary.

Statistics

The primary objective was to identify characteristics that were associated with a clinical pregnancy and live birth after transplantation of ovarian tissue. For that purpose, the study population was divided into two subgroups according to the patient's pregnancy status (yes/no) after transplantation of ovarian tissue. Patients with multiple transplantations were assessed after first transplantation. Both patient subgroups were compared with regard to age at cryopreservation (continuous), age at transplantation (continuous), duration of tissue storage (continuous), patient's disease (categorical, 'breast cancer', 'gynaecological malignancy', 'hodgkin's lymphoma', 'other malignant haematological disease', 'other malignant disease' and 'non-malignant disease'), centre size (categorical; 'small' when <10 transplantations, 'large' when ≥10 performed transplantations), overnight transportation (categorical; yes/no), ovarian tissue activity (= recurrence of regular or irregular menstruation), 12 months after transplantation (categorical; 'active before transplantation', 'yes', 'no'), IVF (categorical; 'yes', 'no') and radiation of the pelvis (categorical; 'yes', 'no'). *t*-tests were performed for continuous characteristics, χ^2-tests or Fisher's exact tests were performed for categorical characteristics.

The pregnancy status in the study population was summarized as pregnancy rate, and its 95% CI was calculated using the Clopper–Pearson method. Pregnancy rates with 95% CIs were also estimated for patients <35 years of age at cryopreservation and those who were older.

The influence of age at cryopreservation on pregnancy after transplantation was further studied using a logistic regression model with pregnancy status ('yes'/'no') as outcome and age at cryopreservation as continuous predictor. The predictor was used as natural cubic spline function to describe possible non-linear effects (Harrell *et al.*, 1988). The corresponding number of degrees of freedom (between one and three) was determined as described previously (Häberle *et al*, 2016). Pregnancy rates depending on age at cryopreservation with corresponding 95% CIs were estimated using this logistic regression model, knowing the CIs might be biased to a certain degree because the logistic regression model was applied to data that had already been used to assess model complexity.

In an exploratory analysis, the effect of centre size on pregnancy rate was studied by calculating the pregnancy rate in each study centre and dividing study centres into five categories according to their numbers of transplantations (1–3, 4–9, 10–19, 20 and more transplantations). For each category, the mean of the pregnancy rates was calculated and shown in a plot.

A secondary objective was to assess the pregnancy rate in patients with multiple transplantations of ovarian tissue when the first transplantation had not been successful. Another secondary objective was to assess the overall pregnancy rate, which is defined as the proportion of patients who became pregnant at least once after one or more transplantations of ovarian tissue.

Live birth rates were studied in a similar way to the analyses of pregnancy rates.

Note that pregnancy rates and live birth rates for which statistical analyses (CIs, tests) were carried out are 'per patient' and not 'per transplantation'. This definition guarantees independence of the observations and allows ordinary statistical tests to be carried out and 95%

CIs to be calculated. No statistical tests or CIs for 'per transplant' were performed.

All the tests were two-sided, and a *P*-value of <0.05 was regarded as statistically significant. *P*-values were not corrected for multiple testing. Calculations were carried out using the R system for statistical computing (version 4.1.1; R Development Core Team, Vienna, Austria, 2021).

Ethics

Approval was obtained from the university's local ethics committee (application number: 192_18 B).

Results

One hundred and ninety-six patients were included in the analysis. The average age of the women was 31.3 years (SD, 5.2; range, 17–44 years) at the time of cryopreservation and 35.9 years (SD, 4.8; range, 23–47 years) at the time of transplantation. Patient's characteristics and summary statistics for study population overall and relative to pregnancy status after first ovarian tissue transplantation are shown in Table I. The characteristics of the age groups at the time of cryopreservation are shown in Table II. Figure 1 presents live birth data and Supplementary Fig. S1 the corresponding pregnancy data.

Pregnancy rates and live birth rates in relation to female age at the time of tissue freezing

Sixty-four of the 196 women became pregnant, representing a pregnancy rate per patient of 32.7% (95% CI, 26.1–39.7%) (Table II). In total, 244 transplantations were performed and 80 pregnancies were achieved.

Patients who became pregnant after the first transplantation were younger at the date of cryopreservation (mean 29.9 years) compared to those without a pregnancy (mean 31.9 years, $P=0.01$). Both patient groups also differed regarding age at the time of transplantation (with pregnancy: mean 34.8 years vs without pregnancy mean 36.4 years; $P=0.02$) (Table I).

In women <35 years at the time of cryopreservation, pregnancy rate after first transplantation was 34.5% (95% CI, 26.7–42.9%) versus 20.4% (95% CI, 10.6–33.5%) in women ≥ 35 years (Table II). Supplementary Figure S1 presents the decreasing pregnancy rate as a continuous function of increasing female age at cryopreservation. The estimated pregnancy rate was 48.1% (95% CI, 32.7–63.8%) at the age of 21 (5th percentile of study population) and 19.4% (95% CI, 11.8–30.2%) at the age of 39 (95th percentile).

Fifty-two out of 196 patients gave birth after first or repeated transplantations, corresponding to an overall live birth rate per patient of 26.5% (95% CI, 20.5–33.3%) (Table II). Among women who became pregnant, pregnancy was achieved by IVF in 38.3%. Percentage of pregnancies that were achieved in spontaneous cycles was 61.7%.

In women <35 years at the time of cryopreservation, live birth rate after first transplantation was 28.2% (95% CI, 20.9–36.3%) versus 16.7% (95% CI, 7.9–29.3%) in women ≥ 35 years (Table II).

Birth rate as a continuous function of age at cryopreservation is shown in Fig. 1, revealing estimated live birth rates of 40.0% (95% CI, 25.5–56.6%) at the age 21 (5th percentile) and 15.7% (95% CI, 9.1–25.9%) at the age of 39 (95th percentile).

First versus repeated transplantation

Sixty of 196 patients became pregnant after the first transplantation, corresponding to a pregnancy rate per first transplantation of 30.6% (95% CI, 24.2–37.6%). Forty-nine out of 196 patients gave birth after the first ovarian tissue transplantation, representing an overall live birth rate per first transplantation of 25.0% (95% CI, 19.1–31.7%) (Table II).

Forty-three patients underwent repeated transplantations. Thirty-nine women underwent a second transplantation and four women even a third transplantation. The characteristics of the cases with first and with repeated transplantations are described in Table III.

Nine of the 43 women had already become pregnant after the first tissue transplantation. Of these 9 women, 5 (55.5%) became pregnant again after repeated transplantation and 4 gave birth (44.4%) (Table III).

Thirty-four of the 43 women did not become pregnant after the first transplantation. Of these 34 women, 4 (11.8%, 95% CI, 3.3–27.5%) became pregnant and 3 (8.8%, 95% CI, 1.9–23.7%) gave birth. These three women were <35 years old at the time of cryopreservation (Table II).

Experience of transplanting centres

Ovarian tissue transplantations were carried out in 26 centres of the FertiPROTEKT network. The total number of transplantations performed per centre varied between 1 and 100 (median, 2) and the number of patients between 1 and 82.

The mean live birth rates relative to centre size defined by the number of ovarian tissue transplantations are shown in Fig. 2. The figure indicate that the live birth rate increases with the size of the centre.

In centres with ≥ 10 transplantations ($n=5$ centres), 46 out of 135 women achieved a pregnancy after first transplantation corresponding to a pregnancy rate of 34.1% (Table I). In centres with <10 transplantations ($n=21$ centres), 14 out of 61 women became pregnant after first transplantation corresponding to a pregnancy rate of 22.9% (Table I, $P=0.12$).

A total of 185 transplantations were performed in centres who had performed ≥ 10 transplantations (Supplementary Table SI).

In centres with ≥ 10 performed transplantations, 65 out of 185 transplantations resulted in a pregnancy and 40 in a live birth, corresponding to a pregnancy rate of 35.1% and a live birth rate of 27.0% per transplantation.

In centres with <10 performed transplantations ($n=21$ centres), 15 out of 59 transplantations resulted in a pregnancy and 11 in a live birth, corresponding to a pregnancy rate of 25.4% and a live birth rate of 18.6% per transplantation.

An influence of the experience of a centre on the association between age at cryopreservation and success rates could not be shown (sensitivity analysis during review process, logistic regression analysis). Age at cryopreservation, duration of tissue storage, overnight transport status, indication for ovarian tissue cryopreservation and pelvic

Table I Patient's characteristics and summary statistics for study population overall and relative to pregnancy status after first ovarian tissue transplantation.

Characteristic		All patients (N = 196)*	Patients without pregnancy (N = 136)	Patients with pregnancy (N = 60)	*P* value
Age at cryopreservation, mean (SD), median (range)		31.3 (5.2) 32 (17, 44)	31.9 (5.2) 32.5 (17, 44)	29.9 (5.2) 31 (17, 39)	0.01
Age at transplantation, mean (SD), median (range)		35.9 (4.8) 37 (23, 46)	36.4 (4.8) 37 (23, 46)	34.8 (4.5) 35.5 (25, 43)	0.02
Duration of tissue storage, mean (SD), median (range)		4.6 (2.7) 4 (0, 15)	4.5 (2.8) 4 (0, 15)	4.9 (2.6) 4 (1, 13)	0.34
Disease, n (%)	Breast cancer	82 (42.1)	55 (40.7)	27 (45.0)	0.64
	Gynecological malignancies	18 (9.2)	14 (10.4)	4 (6.7)	
	Hodgkin's lymphoma	52 (26.7)	33 (24.4)	19 (31.7)	
	Other malignant haematologic diseases	21 (10.8)	16 (11.9)	5 (8.3)	
	Other malignant diseases	17 (8.7)	14 (10.4)	3 (5.0)	
	Non-malignant diseases	5 (2.6)	3 (2.2)	2 (3.3)	
Experience of centres, n (%)	<10 transplantations	61 (31.1)	47 (34.6)	14 (23.3)	0.12
	≥10 transplantations	135 (68.9)	89 (65.4)	46 (76.7)	
Overnight transportation, n (%)	Yes	98 (50.3)	65 (47.8)	33 (55.9)	0.30
	No	97 (49.7)	71 (52.2)	26 (44.1)	
Active ovarian tissue 1 year after transplantation, n (%)	Ovaries still active before transplantation	26 (14.9)	19 (16.4)	7 (12.1)	<0.01
	Yes	114 (65.5)	67 (57.8)	47 (81.0)	
	No	34 (19.5)	30 (25.9)	4 (6.9)	
IVF, n (%)	Yes	40 (20.6)	17 (12.7)	23 (38.3)	<0.0001
	No	154 (79.4)	117 (87.3)	37 (61.7)	
Radiation of the pelvis, n (%)	Yes	17 (8.7)	14 (10.4)	3 (5.0)	0.22
	No	178 (91.3)	121 (89.6)	57 (95.0)	

*Some subgroups do not present 196 women due to missing values: disease (N = 1), overnight transport (N = 1), active ovarian tissue (N = 22), IVF (N = 2) and radiation of the pelvis (N = 1).

Table II Pregnancy and live birth rates overall and after first and repeated ovarian tissue transplantations.

	Age at cryopreservation	Patients n	Clinical pregnancies		Live births	
			Patients n	Rate % (95% CI)	Patients n	Rate % (95% CI)
Overall	All ages	196	64	32.7 (26.1, 39.7)	52	26.5 (20.5, 33.3)
	<35 years	142	52	36.6 (28.7, 45.1)	43	30.3 (22.9, 38.5)
	≥35 years	54	12	22.2 (12.0, 35.6)	9	16.7 (7.9, 29.3)
After first transplantation	All ages	196	60	30.6 (24.2, 37.6)	49	25.0 (19.1, 31.7)
	<35 years	142	49	34.5 (26.7, 42.9)	40	28.2 (20.9, 36.3)
	≥35 years	54	11	20.4 (10.6, 33.5)	9	16.7 (7.9, 29.3)
After repeated transplantations*	All ages	34	4	11.8 (3.3, 27.5)	3	8.8 (1.9, 23.7)
	<35 years	25	3	12.0 (2.5, 31.2)	3	12.0 (2.5, 31.2)
	≥35 years	9	1	11.1 (0.3, 48.2)	0	0.0 (0.0, 33.6)

*If no pregnancy after first transplantation.

irradiation were not different in both less and more experienced centres (Supplementary Table SI).

Further outcomes

Further outcomes are shown in Table I overall and relative to pregnancy status.

Patients with pregnancy after transplantation and those without differs regarding ovarian tissue activity 1 year after transplantation ($P < 0.01$) and IVF status ($P < 0.0001$). The proportion of patients with active tissue 1 year after transplantation and IVF was higher in patients with pregnancy than in those without pregnancy.

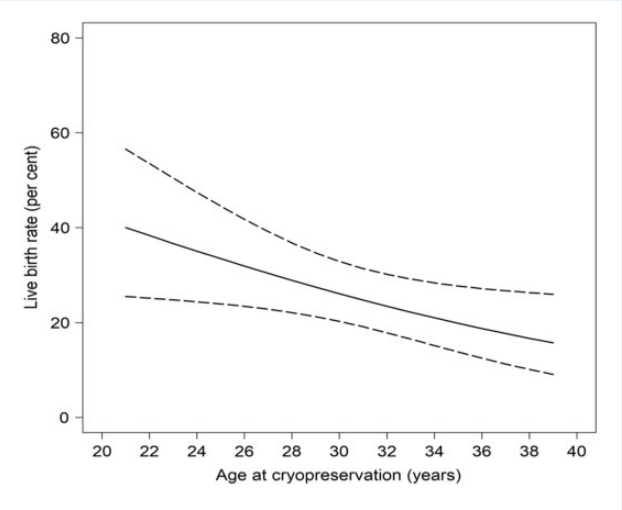

Figure 1. Live birth rate as a continuous function of age at cryopreservation with pointwise 95% confidence intervals (dashed lines), based on logistic regression analyses.

A difference with regard to radiation of the pelvis or overnight transportation could not be shown.

Overnight ovarian tissue transportation at around 4°C before freezing was performed in 98 cases (50.3%). The pregnancy rate after overnight transportation was 33.6% and after direct freezing 26.8%. The result for overnight transportation was confirmed in a sensitivity analysis during the review process that took age at cryopreservation into account as an adjustment variable (logistic regression analysis).

Discussion

Our study revealed a live birth rate per transplanted woman of 26.5%. The success rate was significantly associated with female age at the time of tissue freezing. Success rates were higher in centres experienced in ovarian tissue transplantation and success rates were lower in repeated transplantation when pregnancy did not occur after the first transplantation.

The strength of the study is the large number of transplantations analysed. Furthermore, all transplantations were performed within a network with the same standards regarding tissue freezing, tissue transportation and tissue transplantation.

The weakness is its retrospective design and the heterogeneity of data due to the high number of centres. However, this study reflects a real-life scenario in a country where many centres are involved in tissue freezing and transplantation. Furthermore, this heterogeneity allowed an analysis regarding the success rate in relation to the centre's experience. However, the heterogeneity might also have led to some bias, as more experienced centres might be better organized regarding the follow-up of the patients.

Previous studies on ovarian tissue freezing and transplantation have shown that the success rates differ substantially among centres and

Table III Characteristics of patients with repeated ovarian tissue transplantations with and without pregnancy after the first transplantation.

	Repeated transplantations, with a pregnancy after first transplantation (n = 9 patients)	Repeated transplantations, without a pregnancy after first transplantation (n = 34 patients)
Age at cryopreservation, mean (SD)	31.1 (2.6)	30.4 (5.6)
Age at transplantation, mean (SD)	39.5 (2.6)	35.9 (4.7)
Freezing after overnight transportation, n (%)	5 (55.5)	17 (50.0)
Transplantation by experienced centre (>10 transplantations), n (%)	9 (100)	27 (79.4)
Duration of tissue storage, mean years (SD)	7.1 (2.6)	5.3 (2.7)
Transplantation after breast cancer, n (%)	5 (55.6)	8 (23.5)
Transplantation after lymphoma, n (%)	3 (33.3)	18 (52.9)
Transplantation after other diseases, n (%)	1 (11.1)	8 (23.5)
Radiation of the pelvis, n (%)	0 (0)	2 (5.8)
Tissue activity >1 year, n (%)	7 (100)	26 (76.5)
Pregnancies, n (%)	5 (55.5)	4 (11.8)
Live births, n (%)	4 (44.4)	3 (8.8)

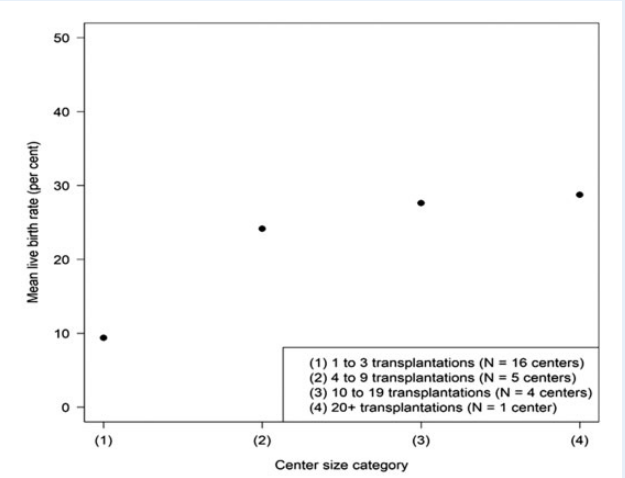

Figure 2. Mean live birth rates relative to centre size categories defined by the number of performed ovarian tissue transplantations.

countries. Shapira *et al.* (2020) selected three centres from three different countries (Israel, Belgium and the USA) which had published high success rates and summarized their data to publish a live birth rate per woman as high as 41.7%.

Others such as van der Ven *et al.* (2016) (*Ferti*PROTEKT network: Germany, Austria, Switzerland) and Dolmans *et al.* (2021) (five European countries or networks: *Ferti*PROTEKT, Denmark, Spain, France, Belgium) summarized the data of several national cohorts, irrespective of their individual success rates, and published 12 live births rates in 49 transplanted women (live birth rate per women: 24.5%) (van der Ven *et al.*, 2016) and 95 live births rates in 285 women (26.5%) (Dolmans *et al.*, 2021), respectively. Furthermore, in a recent meta-analysis by Pacheco *et al.* (2017), the global live birth rate was 37.7% per woman, with at least 63.9% of recipients having ovarian function of >6 months duration. The live birth plus ongoing (LB + OG) pregnancy rate from this meta-analysis was updated 2022 by Oktay *et al.* including only studies that reported the total number of transplant recipients to calculate the LB + OG pregnancy rate. The LB + OG pregnancy rate per woman receiving a transplant was 25% (115 LB + OG in 460 recipients).

Shapira *et al.* (2020) presented data which can be interpreted as 'the best possible success rate', whereas van der Ven *et al.* (2016), Dolmans *et al.* (2021) and Oktay *et al.* (2022) as 'the realistic success rate'. In other words, Shapira *et al.* demonstrated what is possible under highly optimized conditions, whereas van der Ven *et al.* and Dolmans *et al.* demonstrated success rates under less optimized conditions, such as inclusion of many centres with different experience in the process of tissue freezing and transplantation.

Our study adds a large data series to the already published cohorts, representing the second scenario, 'the realistic success rates'. Accordingly, the presented success rates are very similar to those published by van der Ven *et al.* (2016) and Dolmans *et al.* (2021).

Our study confirms that freezing and transplantation of ovarian tissue is a realistic option, as at least one in four transplanted women achieve a live birth. However, our study also addresses the question of which factors are associated with high success rates and therefore if indications can be defined to increase the success rate.

Previous studies such as van der Ven *et al.* (2016) and Dolmans *et al.* (2021) have already shown that the success rates are negatively associated with increasing age at the time of tissue freezing. Our study confirmed these results and demonstrated much higher live birth rates in women <35 years (30.3%) versus women ≥35 years (16.7%) at the time of tissue freezing. Cryopreservation of ovarian tissue in women over 35 years of age is generally not recommended but has been performed in individual cases after informed consent in the FertiPROTEKT network. The maximum age for cryopreservation in conjunction with live birth was 38 years at the time of cryopreservation in this study. Due to the high number of transplantations, we could also show that the success rate does not decrease abruptly at the age of 35 years, but rather smoothly over a period of many years, questioning the discussed cut-off of 35 years for ovarian tissue freezing (von Wolff, 2020b).

Previous studies have already tried to address the success of first versus repeated transplantations (Jensen *et al.*, 2015; van der Ven *et al.*, 2016), but without clear conclusions. We also addressed this topic and found much higher live birth rates in women after first (25.0%) versus after repeated transplantations (8.8%). This data does not allow the conclusion to be drawn that more or even all tissue should be used for the first transplantation. In our cohort, three of the nine women who had become pregnant after the first transplantation delivered a baby after repeated transplantations. However, our data do allow the conclusion that repeated transplantation after a failed first transplantation need to be indicated with care. In our cohort, only 3 of the 34 women (8.8%) who had not become pregnant after the first transplantation did deliver a baby after repeated transplantations. This might be especially relevant if, as in our cohort, only 50% of one ovary had been frozen.

The third new aspect provided by our study is the relevance of the experience of the transplanting centre. We demonstrated that success rates were higher in centres with ≥10 transplantations. This data opens the discussion as to whether transplantation should only be performed by experienced centres. However, our findings should be interpreted with care. First, the differences are not significant and it is unclear whether the higher pregnancy rates are due to better surgical performance of the experienced centres, or better expertise in freezing and thawing ovarian tissue, or biased by the different intensity of follow-up care in the centres. Furthermore, it should be noted that the comparison of the results of centres with <10 or ≥10 transplants performed is based on the number of transplants and not on the number of transplanted patients.

Still, even if subperitoneal transplantation of ovarian tissue, as performed in 87.5% of our cohort, can be regarded as being not very difficult, it could be the complete package of tissue thawing, tissue preparation, transplantation and post-transplantation care which contributes to the success rate. Therefore, we would like to suggest that only centres which have optimized all these processes should transplant ovarian tissue.

We also studied the impact of tissue overnight transportation on the success rates. We did not find an association, which is in line with

the study by Rosendahl et al. (2011) and Liebenthron et al. (2019). They had already demonstrated high live birth rates after transportation at around 4°C for 5 h (Jensen et al., 2017) or even up for to 24 h (Liebenthron et al., 2019).

In this study, 37% of women achieved pregnancy through IVF treatment, while 63% became pregnant spontaneously. A total of 40 women underwent IVF treatment, of whom 23 became pregnant (57.5%), while 37 of 154 (24.0%) women became pregnant spontaneously. These data confirm that both spontaneous and IVF fertilization are possible after ovarian tissue transplantation. However, the registry does not allow specific pregnancy rates per IVF treatment. There is limited information on IVF cycles after ovarian tissue transplantation. There are reports indicating a relatively low response (Greve et al., 2012; Dolmans et al., 2021), while other papers report high follicular yield and good pregnancy rates (Oktay et al., 2022). Overall we believe that some women may benefit from immediate IVF treatment with oocyte retrieval and cryopreservation after transplantation, as tissue activity is time-limited. However much more research and development is required to optimize methods and define age limits and the most appropriate stimulation regimens (Dolmans et al., 2021).

Pelvic irradiation appears to significantly decrease the likelihood of a successful pregnancy (Teh et al., 2014). In this study, no difference in pregnancy rates was observed after pelvic irradiation. However, only 17 (8.7%) women had a history of pelvic irradiation and pregnancy occurred in only three of them, with only one woman with B-cell lymphoma giving birth. Data on radiation fields and dose were not reported in the registry. Therefore, the results here should be interpreted with caution and ovarian tissue transplantation after high-dose pelvic irradiation, as in anal and cervical carcinoma, should be carefully considered because of the low chance of success and obstetric risks (Dolmans et al., 2021). Our study and previously published studies suggest that if indications for freezing of ovarian tissue and transplantation are strict and if transplantations are performed by highly specialized centres, overall success rates can probably be increased, possibly up to around 40% as published by Shapira et al. (2020). Such success rates would be similar to freezing of oocytes (Cobo et al., 2018; Diaz-Garcia et al., 2018).

Strict indications would also increase the far too low rate of women who return for tissue transplantation. Return rates have been reported to be between 2.9% (von Wolff, 2020a) and 19% (Kristensen et al., 2021).

Furthermore, various strategies are currently being investigated to improve the quality of transplanted ovarian tissue and the clinical outcomes of ovarian tissue transplantation (Roness et al., 2019; Takae et al., 2019; Marin et al., 2020). One approach is ovarian tissue transplantation with robotic surgery and a neovascularizing human extracellular matrix scaffold. Using this technique, it has recently been shown that it is possible to prolong graft life and achieve a high recovery of ovarian function, even when the tissue has been cryopreserved after chemotherapy (Oktay et al., 2022).

In conclusion, according to our study, one in four transplanted women become pregnant and deliver at least one baby. This success rate is higher in women who had tissue frozen at a young age. Furthermore, the success might be even higher if the transplantation is performed by a highly experienced centre which focuses on the first transplantation. Therefore, ovarian tissue freezing and transplantation have a high potential to generate pregnancies and live births in young women, but apparently only if the indication, logistics and surgical techniques are optimized.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Authors' roles

Study concept and design: L.L., J.B.-L., R.D., M.v.W. and M.W.B. Acquisition of data: L.L., J.B.-L., R.D., W.v.M., A.G., M.K., N.S. and J.S.K. Analysis and interpretation of data: L.L., J.B.-L., R.D., L.H., W.v.M. and M.W.B. Drafting of the manuscript: L.L. and W.v.M. Critical revision of the manuscript for important intellectual content: J.B.-L., R.D., L.H., M.W.B., A.G., M.K., N.S. and J.S.K. Statistical analysis: L.H. Study supervision: R.D., W.v.M. and M.W.B.

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Conflict of interest

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