Effect of first line cancer treatment on the ovarian reserve and follicular density in girls under the age of 18 years

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Objective: To study the impact of first-line antineoplastic treatment on ovarian reserve in young girls returning for ovarian tissue cryopreservation (OTC) in connection with a relapse.
Design: Retrospective case-control study.
Setting: University hospitals.
Patient(s): Sixty-three girls under the age of 18 years who underwent OTC before (group 1: 31 patients) and after (group 2: 32 patients) their initial cancer treatment.
Intervention(s): None.
Main Outcome Measure(s): Follicular densities (follicles/mm^3) measured from an ovarian cortical biopsy before OTC. The ovarian volume (mL) of entire ovaries excised for OTC was also monitored.
Result(s): There was no statistically significant difference in the mean age or follicular density between groups 1 and 2 (334 $\pm$ 476/mm^3 vs. 327 $\pm$ 756/mm^3). In contrast, the ovarian volume and total number of ovarian cortex chips cryopreserved were statistically significantly lower in patients who received gonadotoxic treatment before OTC (mean $\pm$ standard deviation [SD]: ovarian volume, 5.3 $\pm$ 3.1 mL vs. 2.9 $\pm$ 2.1 mL, respectively; number of cortex chips: 21.3 $\pm$ 8.1 vs. 15.2 $\pm$ 7.1, respectively). The reduction in the estimated ovarian reserve ranged from 10% to 20% in children to around 30% in adolescent girls (>10 years).
Conclusion(s): Girls under the age of 10 tolerate a gonadotoxic insult better than adolescents, who may experience up to a 30% reduction in the ovarian reserve via first-line gonadotoxic treatment, which at present is considered to have little effect on the follicle pool. This information will improve counseling of young female cancer patients in deciding whether to undergo fertility preservation treatment. (Fertil Steril® 2016;106:1757–62. ©2016 by American Society for Reproductive Medicine.)
Key Words: Fertility preservation, follicle density, ovarian tissue cryopreservation, ovarian volume

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During the last 50 years, cancer diagnoses in children and adolescents have progressed from being a serious potentially fatal disease to a most often curable disease. The 5-year survival for all cancer types is estimated to be more than 80% in both children and adolescents (1). Furthermore, the late-effect mortality from any cause has statistically significantly decreased across the last decades among 5-year survivors of childhood cancer according to the Childhood Cancer Survivor Study (2). This success comes from a remarkable development of effective therapeutic regimens, including alkylating agent-based chemotherapy and radiotherapy.

This progress has created an awareness of quality of life aspects after cancer, highlighting that successful

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treatment may compromise fertility after recovery (3). The pool of ovarian follicles that constitute the reproductive potential of a girl may be severely reduced or disappear as a consequence of the treatment required (4). In prepubertal girls the only available option to preserve fertility is to cryopreserve ovarian tissue (5, 6). If ovarian activity is destroyed and premature ovarian insufficiency (POI) occurs in young female survivors, cortical ovarian tissue might be transplanted to restore ovarian function for fertility purposes (7, 8). Recently, the first child born after transplantation of ovarian tissue that was harvested before menarche but after puberty was reported (9).

However, it is often difficult to decide whether it is necessary for a young girl with cancer to undergo an invasive procedure to obtain ovarian tissue for fertility preservation. The fertility preservation intervention is recommended when there is an estimated risk of POI exceeding 50% (10, 11). Ovaries from girls and young women contain a very high number of follicles and may tolerate a relatively high gonadotoxic insult without losing all follicles (12). In most cases treatment is initiated with low-risk regimens, but if more aggressive treatment is needed, harvesting ovarian tissue for fertility preservation will be considered. The question thus arises as to whether ovarian tissue cryopreservation (OTC) should be considered in connection with a first-line treatment such as ABVD (Adriamycin, bleomycin, vinblastine, dacarbazine), which is often considered to cause a relatively mild gonadotoxic insult. Thus, the question is: What is the potential gonadotoxic insult caused by a first-line cancer treatment on the fertility potential in prepubertal and adolescent girls? To answer this question we evaluated the follicular density and ovarian volume in our cohort of young girls below the age of 18 years with respect to whether they had received gonadotoxic treatment before OTC.

MATERIALS AND METHODS

Patients

This retrospective study included a total of 63 girls younger than 18 years (range: 1.5–17.9 years) with a cancer diagnosis who had been referred to one of the three centers that participate in the Danish program for fertility preservation by OTC between the years 2002 and 2014. The number of patients who had not received chemotherapy before oophorectomy was 31 (group 1), and a total of 32 patients (group 2) had received low-risk gonadotoxic treatment before ovarian excision. All the patients in group 2 had been treated for an original oncologic diagnosis, had experienced a relapse, and had undergone OTC before further treatment. Patients were only included if a biopsy sample of their ovarian cortex was spared for histology in connection with OTC.

Procedure

The ovarian cortex was isolated by manual dissection and cut into pieces of approximately 5 × 5 mm and 1 mm thickness and frozen by slow-freezing technique as previously described elsewhere (13, 14). A small ovarian cortical biopsy (≈2 × 2 × 1 mm) is routinely taken for histologic examination before freezing. The piece was processed for histology, cut into 30-μm sections, and stained with periodic-acid Schiff reagents and Mayer hematoxylin. The follicular density, follicles per mm^3, was calculated by counting all types of follicles in every second section as previously described elsewhere (15). Because one entire ovary was removed, the ovarian volume was recorded by weighing the tissue before preparation for cryopreservation. The density of ovarian tissue has previously been determined to be 1 g/mL (16) using tissue weight and volume calculated by insertion in 0.9% NaCl solution. The ovarian surface area was calculated assuming that the ovarian volume represented a spherical structure.

Statistics

Microsoft Excel version 14.6.1 was used to analyze the data. The data for each variable for the pretreated and nonpretreated groups were symmetrically distributed with similar variances between groups 1 and 2, hence Student's *t*-test assuming equal variance was used to compare the between-group means of follicular density, ovarian volume, and number of ovarian cortex pieces (17). Age-adjusted comparisons were not performed because of the similar age characteristics (mean, median, interquartile range, range) between the two groups. $P<.05$ was considered statistically significant throughout the study. Quadratic regression curves were fitted to the data for both groups to visualize the similarities and differences reported (Figs. 1 and 2) and to estimate the age-related loss in ovarian reserve after treatment (Table 1).

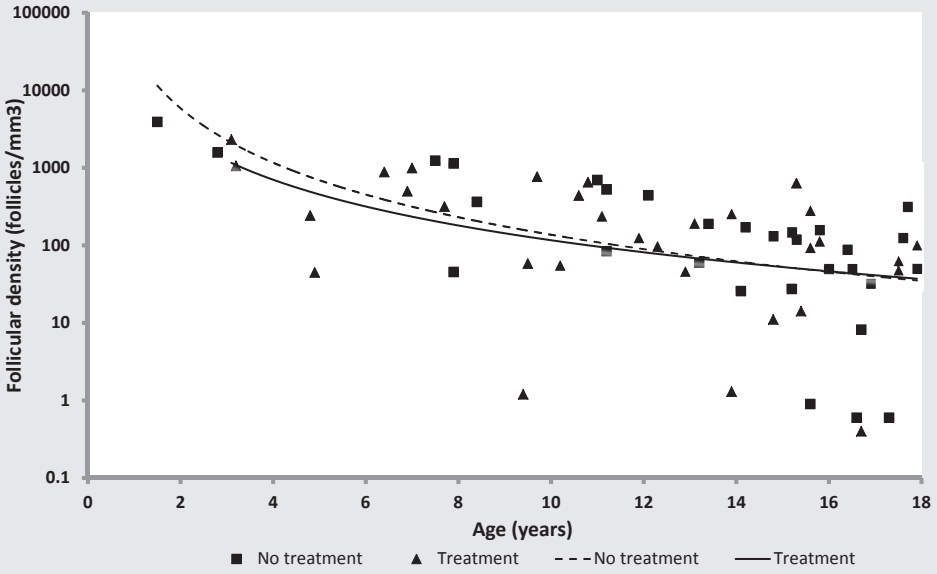
The project of ovarian tissue cryopreservation was approved by the ethics committee of Copenhagen and Frederiksberg (H-2-2001-044). The storage and collection of patient data were approved by the Ministry of Health (J. no. 30-1372) and by the Danish authorities to comply with European Union tissue directives.

RESULTS

There was no statistically significant difference in mean age (±standard deviation [SD]) between groups 1 and 2 (13.2 ± 4.1 vs. 11.6 ± 4.3 years, $P=.19$. Cancer diagnoses for patients in the two groups are listed in Table 2. In group 1, the most frequent diagnoses were Hodgkin lymphoma (n = 8) and Ewing sarcoma (n = 6) whereas hematologic malignancies (acute lymphoblastic leukemia and acute myeloid leukemia, n = 14) were the most frequent in group 2. The chemotherapy regimens used in group 2 were all classified as having a low or moderate gonadotoxic impact as, for instance, low-dose alkylating agents. However, it has not been possible to recover information on the actual cancer treatments administered before excision of ovarian tissue. In all 63 patients a one-sided oophorectomy was performed to harvest ovarian tissue. No surgical complications were reported in connection with the oophorectomy.

Patients with leukemia received chemotherapy for relapse close to the OTC procedure (i.e., <1 month), while the patients with Ewing sarcoma/other sarcoma and other cancers received their last chemotherapy at months to years before OTC, depending on the time of relapse. However, these

FIGURE 1



Follicular density in ovarian cortex. No statistically significant difference was found in follicular density in the ovarian cortex from patients who did or did not receive first-line antineoplastic treatment. The lines are quadratic best-fit to the data for the two groups.

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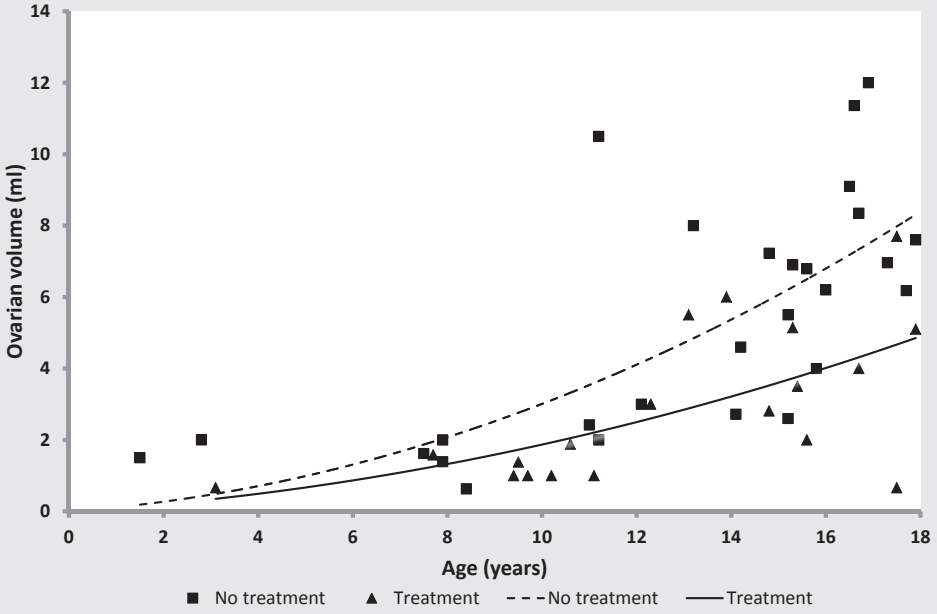
patients had in some cases initiated chemotherapy of their relapse just before OTC.

There was no statistically significant difference in follicular density in the ovarian cortex between patients who received first-line chemotherapy before OTC and patients who did not (334 ± 476/mm^3 vs. 327 ± 756/mm^3, $P>.10$) (Fig. 1). In contrast, the ovarian volume was statistically significantly higher in group 1 than in group 2 (mean ± SD: 5.3 ± 3.3 vs. 2.9 ± 2.1 mL, $P<.05$) (Fig. 2). Similarly, the total number of ovarian cortex chips cryopreserved was

FIGURE 2



Ovarian volume. Ovarian volume was statistically significantly reduced by previous antineoplastic treatment as compared with those who did not receive pretreatment ($P<.05$). The lines are quadratic best-fit to the data for the two groups.

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TABLE 1

The estimated ovarian volume and the estimated ovarian reserve after gonadotoxic treatment in girls younger than 18 years.

	Ovarian volume (mL)		Ovarian surface area (mm^3)		Estimated ovarian reserve (%) after
Age (y)	Group 1	Group 2	Group 1	Group 2	treatment
2	0.4	0.34	2.63	2.36	90
4	0.79	0.58	4.14	3.37	81
6	1.34	0.91	5.89	4.55	77
8	2.08	1.34	7.90	5.89	75
10	3	1.87	10.08	7.36	73
12	4.09	2.49	12.40	8.90	72
14	5.36	3.21	14.84	10.55	71
16	6.8	4.02	17.40	12.25	70
18	8.43	4.93	20.07	14.04	70

Note: Group 1: patients no treatment; group 2: patients receiving chemotherapy before OTC. Volumes are obtained from the line of best fit to our data for the two groups (Fig. 2); surface areas are calculated from volumes; ovarian reserve for each age is estimated as group 2 surface area as a percentage of group 1 surface area. OTC = ovarian tissue cryopreservation.

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higher in patients who did not receive cancer treatment before ovary removal (mean ± SD: 21.3 ± 8.1 vs. 15.2 ± 7.1, $P<.05$; Supplemental Fig. 1, available online). The ovarian surface area was calculated assuming that the volume represents a spherical structure. The pool of primordial follicles is situated approximately 1 mm below the ovarian surface epithelium; the surface area thus represents the ovarian pool. Further, this applies to both groups because the average estimated follicular density was similar between the two groups. After cancer treatment the estimated ovarian surface area was reduced by around 10% in young girls and around 30% in adolescent girls (Table 1).

DISCUSSION

Our current study found no statistically significant differences in terms of follicular density between young girls who received first-line chemotherapy before OTC and those who did not. On the other hand, the ovarian volume and the total number of ovarian cortex pieces were statistically significantly reduced by cancer treatment received before OTC. Thus, the ovarian reserve and the future fertility potential, could—depending on age—be reduced by the first-line chemotherapy by almost 30% in girls 10 to 18 years of age.

In this study the patients who received low-risk treatment initially did not receive fertility preservation, but they were referred to OTC after a relapse that required intensive cancer treatment with high gonadotoxicity, which justified the OTC. The cryopreservation process of freezing and transplantation of ovarian tissue is far from efficient—only a fraction of the transplanted follicles survive the entire process and become available to the patient. However, young girls lose only an average of 10% of their pool, which is a low reduction and may be without long-term consequences for fertility. Some adolescent girls may lose around 30% of the pool of follicles; they could in some cases be considered differently and would have benefitted from having had OTC performed initially rather than after gonadotoxic treatment with only 70% of the original pool of follicles left, as this study suggests. However, the fact that a relapse would occur was unforeseen in the first place. Our new information provides potential support for the patient and her parents. The information may qualify the difficult decision on whether to offer OTC to patients undergoing low-risk cancer treatments or to only offer it to patients who are undergoing intensified chemotherapy.

We acknowledge that our study has limitations. The first-line treatments for group 2 are not homogeneous, although they are all estimated to be mildly or moderately gonadotoxic. All acute leukemia cases are in group 2, meaning that specific comparisons involving acute lymphoblastic leukemia and acute myeloid leukemia are not available from our data. The measurement of antimüllerian hormone in both groups would have made a positive contribution to our analyses and comparisons.

In light of the increased long-term survival of childhood cancer, counseling about fertility preservation is now recommended to be offered to young patients and their parents before any cancer treatment (18). In girls and young women in whom ovarian stimulation for oocyte/embryo cryopreservation is considered inappropriate, OTC is the only option available. A substantial heterogeneity of inclusion criteria exists, not only worldwide but also within single countries, showing that the selection of patients for OTC is a new emerging area where actual clinical experience is scarce, especially considering the effect of transplantation.

The procedure is usually offered when there is a high risk of POI (>50%). However, in one of the largest cohorts of OTC in girls younger than 16 years, Jadoul et al. (19) demonstrated that it is difficult to estimate the risk of infertility, whereas Wallace et al. (11, 20) had success in predicting the gonadotoxic insult by a given cancer treatment. It is possible to define treatment regimens as low, medium, and high risk for ovarian toxicity, but disease evolution is never totally predictable; currently, an individual assessment is required in each individual case (19–21). An interesting avenue of future investigation based on similar data would be the comparison of ovarian characteristics after intensive chemotherapy for Ewing sarcoma (with ifosfamide) as opposed to treatment for acute lymphoblastic leukemia with much less exposure to alkylating agents.

TABLE 2

Type of cancer diagnosis in girls younger than 18 years old who underwent ovarian tissue cryopreservation with or without prior gonadotoxic treatment.

Diagnosis	No treatment, n	Chemotherapy before OTC, n
ALL or AML	0	14
Aplastic anemia	3	0
Ewing sarcoma/other sarcoma	11	8
Morbus Hodgkin	8	3
Myelodysplastic syndrome	2	0
Other cancers	7	7
Total	31	32

Note: ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; OTC = ovarian tissue cryopreservation.

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Clinically the consequences of an up to 30% reduction of the ovarian reserve in young girls are not known. It is likely that the fertility potential is not significantly reduced in perhaps the first decades of life, but the risk of POI and infertility is related to the different treatment regimens. However, to preserve fertility later in life, oocytes or embryos can be cryostored when there still is a follicular reserve after recovery. Of course this requires additional treatment, which potentially could have been avoided.

Further, very little is known about the prepubertal ovary and the mechanisms occurring during the transition from childhood through puberty to adulthood. In the young ovary, the follicular density is higher (15, 22), and follicles in early stages of development are present both in the cortex and in the medulla (23, 24). A recent report showed that ovarian tissue from prepubertal girls contains a large population of abnormal primordial follicles that are lost in adolescence (25). Moreover, immature follicles collected from prepubertal ovarian tissue may have a more limited capability of follicular development than those retrieved from adult tissue (25–28).

Similarly, data about ovarian volume and follicle distribution are very limited in young girls and adolescents. According to a normative model, the ovarian volume enlargement occurs through childhood and adolescence to reach the maximum volume at 20 years of age, thereafter declining toward menopause and beyond (29). With respect to this model our nontreated volumes are close to the predicted values, and the treated volumes are substantially smaller. Antineoplastic drugs generally affect the growing follicles, but are also able to raise the recruitment of nongrowing follicles and damage the ovarian vascularization in the stromal tissue with a detrimental effect on the ovarian reserve (30).

These observations obviously make it even more difficult to evaluate the mechanisms of gonadotoxicity in young and adolescent girls and make the actual estimations of the gonadotoxic insult in our study important. Further, it is interesting to note that the follicular density remained similar between the two groups (with densities for both groups close to predicted values from a normative model) (31) whereas the ovarian volume was reduced in the treated group. Perhaps the follicular density and overall three-dimensional environment are important for determining initiation of follicular growth and recruitment of interstitial cells to theca cell layer (32).

Patients in group 2 who received cancer treatment before OTC in the majority of cases had a diagnosis of leukemia. However, there were no leukemic patients in group 1, and it was not possible to directly evaluate the gonadotoxic insult by the pretreatment given to leukemic patients. However, the pretreatment given to leukemic patients is usually not considered to exert a strong gonadotoxic effect. This may be different with Ewing and other types of sarcoma, where pretreatment may include alkylating agents. However, there was no statistically significant difference between the follicular density of patients who did or did not receive pretreatment with this diagnosis. There is probably considerable interindividual variations, and a larger data set is possibly required to unravel to potential differences in the gonadotoxic insult caused by specific regimes.

In girls under the age of 10 years, first-line cancer treatment does not compromise the ovarian reserve by more than 10%. In contrast, adolescent girls between 11 and 18 years may experience an estimated reduction of 30% of their ovarian reserve. The precise long-term consequences of having a 30% reduced ovarian reserve are not known today, but the information is important in the counseling of the young patients and their parents and to determine whether fertility preservation should be performed.

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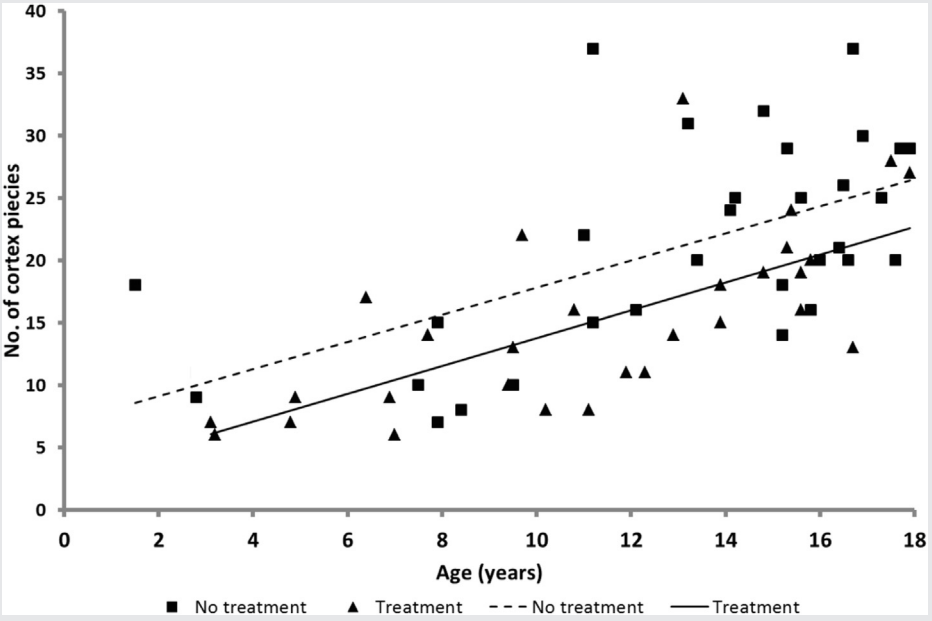
REFERENCES

1. Gatta G, Zigon G, Capocaccia R, Coebergh JW, Desandes E, Kaatsch P, et al. Survival of European children and young adults with cancer diagnosed 1995–2002. Eur J Cancer 2009;45:992–1005.
2. Armstrong G, Chen Y, Yasui Y, Leisenring W, Gibson TM, Mertens AC, et al. Reduction in late mortality among 5-year survivors of childhood cancer. N Engl J Med 2016;374:833–42.
3. Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, et al. Chronic health conditions in adult survivors of childhood cancer. N Engl J Med 2006;355:1572–82.
4. Barton SE, Najita JS, Ginsburg ES, Leisenring WM, Stovall M, Weathers RE, et al. Infertility, infertility treatment, and achievement of pregnancy in female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study cohort. Lancet Oncol 2013;14:873–81.
5. Schmidt KT, Larsen EC, Andersen CY, Andersen AN. Risk of ovarian failure and fertility preserving methods in girls and adolescents with a malignant disease. BJOG 2010;117:163–74.
6. Practice Committee of American Society for Reproductive Medicine. Ovarian tissue cryopreservation: a committee opinion. Fertil Steril 2014;101:1237–43.
7. Donnez J, Dolmans MM. Ovarian cortex transplantation: 60 reported live births brings the success and worldwide expansion of the technique towards routine clinical practice. J Assist Reprod Genet 2015;32:1167–70.
8. Macklon KT, Jensen AK, Loft A, Ernst E, Andersen CY. Treatment history and outcome of 24 deliveries worldwide after autotransplantation of cryopreserved ovarian tissue, including two new Danish deliveries years after autotransplantation. J Assist Reprod Genet 2014;31:1557–64.
9. Demeestere I, Simon P, Dedeken L, Moffa F, Tsepelidis S, Brachet C, et al. Live birth after autograft of ovarian tissue cryopreserved during childhood. Hum Reprod 2015;30:2107–9.
10. Jensen AK, Kristensen SG, Macklon KT, Jeppesen JV, Fedder J, Ernst E, et al. Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. Hum Reprod 2015;30:2838–45.
11. Wallace WH, Kelsey TW, Anderson RA. Fertility preservation in pre-pubertal girls with cancer: the role of ovarian tissue cryopreservation. Fertil Steril 2016;105:6–12.
12. Anderson RA, Mitchell RT, Kelsey TW, Spears N, Telfer EE, Wallace WH. Cancer treatment and gonadal function: experimental and established strategies for fertility preservation in children and young adults. Lancet Diabetes Endocrinol 2015;3:556–67.
13. Rosendahl M, Andersen CY, Ernst E, Westergaard LG, Rasmussen PE, Loft A, et al. Ovarian function after removal of an entire ovary for cryopreservation of pieces of cortex prior to gonadotoxic treatment: a follow-up study. Hum Reprod 2008;23:2475–83.
14. Rosendahl M, Schmidt KT, Ernst E, Rasmussen PE, Loft A, Byskov AG, et al. Cryopreservation of ovarian tissue for a decade in Denmark: a view of the technique. Reprod Biomed Online 2011;22:162–71.
15. Schmidt KL, Byskov AG, Nyboe Andersen A, Müller J, Yding Andersen C. Density and distribution of primordial follicles in single pieces of cortex

from 21 patients and in individual pieces of cortex from three entire human ovaries. Hum Reprod 2003;18:1158–64.
16. Rosendahl M, Ernst E, Rasmussen PE, Andersen CY. True ovarian volume is underestimated by two-dimensional transvaginal ultrasound measurement. Fertil Steril 2010;93:995–8.
17. Lumley T, Diehr P, Emerson S, Chen L. The importance of the normality assumption in large public health data sets. Annu Rev Public Health 2002; 23:151–69.
18. Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, et al. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 2013;31:2500–10.
19. Jadoul P, Dolmans MM, Donnez J. Fertility preservation in girls during childhood: is it feasible, efficient and safe and to whom should it be proposed? Hum Reprod Update 2010;16:617–30.
20. Wallace WH, Smith AG, Kelsey TW, Edgar AE, Anderson RA. Fertility preservation for girls and young women with cancer: population-based validation of criteria for ovarian tissue cryopreservation. Lancet Oncol 2014;15: 1129–36.
21. Nielsen SN, Andersen AN, Schmidt KT, Rechnitzer C, Schmiegelow K, Bentzen JG, Larsen EC. A 10-year follow up of reproductive function in women treated for childhood cancer. Reprod Biomed Online 2013;27: 192–200.
22. Hansen KR, Knowlton NS, Thyer AC, Charleston JS, Soules MR, Klein NA. A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. Hum Reprod 2008;23:699–708.
23. Peters H, Byskov AG, Grinsted J. Follicular growth in fetal and prepubertal ovaries of humans and other primates. Clin Endocrinol Metab 1978;7: 469–85.
24. Kristensen SG, Rasmussen A, Byskov AG, Andersen CY. Isolation of preantral follicles from human ovarian medulla tissue. Hum Reprod 2011;26: 157–66.
25. Anderson RA, McLaughlin M, Wallace WH, Albertini DF, Telfer EE. The immature human ovary shows loss of abnormal follicles and increasing follicle developmental competence through childhood and adolescence. Hum Reprod 2014;29:97–106.
26. Revel A, Revel-Vilk S, Aizenman E, Porat-Katz A, Safran A, Ben-Meir A, et al. At what age can human oocytes be obtained? Fertil Steril 2009;92: 458–63.
27. Fasano G, Moffa F, Dechène J, Englert Y, Demeestere I. Vitrification of in vitro matured oocytes collected from antral follicles at the time of ovarian tissue cryopreservation. Reprod Biol Endocrinol 2011;9:150.
28. Asadi Azarbaijani B, Sheikhi M, Oskam IC, Nurmio M, Laine T, Tinkanen H, et al. Effect of previous chemotherapy on the quality of cryopreserved human ovarian tissue in vitro. PLoS One 2015;10:e0133985.
29. Kelsey TW, Dodwell SK, Wilkinson AG, Greve T, Andersen CY, Anderson RA, et al. Ovarian volume throughout life: a validated normative model. PLoS One 2013;8:e71465.
30. Morgan S, Anderson RA, Gourley C, Wallace WH, Spears N. How do chemotherapeutic agents damage the ovary? Hum Reprod Update 2012;18:525–35.
31. McLaughlin M, Kelsey TW, Wallace WH, Anderson RA, Telfer EE. An externally validated age-related model of mean follicle density in the cortex of the human ovary. J Assist Reprod Genet 2015;32:1089–95.
32. Da Silva-Buttkus P, Marcelli G, Franks S, Stark J, Hardy K. Inferring biological mechanisms from spatial analysis: prediction of a local inhibitor in the ovary. Proc Natl Acad Sci USA 2009;106:456–61.

SUPPLEMENTAL FIGURE 1



Number of ovarian cortex chips cryopreserved to preserve fertility. The number of cryopreserved pieces of cortex was statistically significantly lower in patients who underwent cancer treatment before removal of one ovary compared to those who did not receive pretreatment ($P<.05$).

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