Evaluation of a human ovarian follicle isolation technique to obtain disease-free follicle suspensions before safely grafting to cancer patients

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Objective: To evaluate the safety of our follicle isolation procedure in a model of ovarian tissue artificially contaminated with cancer cells, then to improve the procedure to effectively eliminate malignant cells from follicle suspensions without altering viability.
Design: Prospective experimental study.
Setting: Gynecology research unit in a university hospital.
Patient(s): Ten women undergoing laparoscopy for benign gynecologic disease.
Intervention(s): Follicle isolation from ovarian tissue artificially contaminated with marked fluorescent leukemic cells, either by the usual pickup technique without further treatment (group 1) or by washing three times after pickup (group 2).
Main Outcome Measure(s): Evidence of leukemic cells in follicle suspensions using fluorescence microscopy and quantitative real-time polymerase chain reaction, and analysis of follicle viability.
Result(s): In group 1, 196 leukemic cells were detected by fluorescence microscopy out of 499 follicles retrieved, while just one leukemic cell was found among 772 follicles after three washes. The BCR-ABL fusion transcript was detected when at least 19 cells were present in follicle suspensions; four samples were positive in group 1, and all were negative in group 2. Follicle viability was similar in both groups (95.6% vs. 96.4%).
Conclusion(s): Cancer cells could inadvertently be picked up with isolated follicles in case of malignant contamination of ovarian tissue. A simple purging procedure consisting of three washes proved effective for eliminating leukemic cells while maintaining good follicle viability. (Fertil Steril® 2015;104:672–80. ©2015 by American Society for Reproductive Medicine.)
Key Words: Follicle isolation, leukemia, malignant cell purging, minimal disseminated disease, ovarian follicles, ovarian tissue cryopreservation

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Advances in cancer treatments and aggressive chemotherapy and radiotherapy have led to an increase in survival and life expectancy of young cancer patients. Indeed, the 5-year relative survival rate in children, adolescents, and young adults increased from approximately 60% in 1975 to 82%–86% in 2009 (1). As of January 1, 2010, there were approximately 380,000 survivors of childhood

Received February 11, 2015; revised and accepted May 18, 2015; published online June 19, 2015.
M.S. has nothing to disclose. K.S. has nothing to disclose. C.A.A. has nothing to disclose. P.S. has nothing to disclose. J.D. has received personal fees for scientific presentations from Serrono, MSD, and Ferring. M.-M.D. has nothing to disclose.
Supported by grants from the Fonds National de la Recherche Scientifique de Belgique (5/4/150/5 and 7.4518.12F), Fonds Spéciaux de Recherche, Fondation Saint Luc, and Foundation Against Cancer, and donations from Mr. Pietro Ferrero, Baron Frère, and Viscount Philippe de Spoelberch.
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Fertility and Sterility® Vol. 104, No. 3, September 2015 0015-0282/$36.00
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http://dx.doi.org/10.1016/j.fertnstert.2015.05.021

and adolescent cancer (diagnosed at age 0 to 19 years) still alive in the United States (2).Unfortunately, in many women these life-saving treatments lead to early menopause and subsequent infertility, depending on the age of the patient, her follicular reserve, and the type and dose of drugs used (3–6). Embryo or oocyte cryopreservation may be performed when chemotherapy can be delayed by a minimum of 2–3 weeks, the time required for ovarian stimulation (7, 8). These options are not, however, applicable to prepubertal girls or patients requiring immediate chemotherapy (8–11). For these young women, ovarian tissue cryopreservation followed by orthotopic transplantation has emerged as a promising approach to safeguard fertility, resulting in restoration of endocrine function in most patients and more than 40 live births to date (10,12–15).

Leukemia is the most frequently encountered malignancy in children (0–14 years) representing 26% of all tumors diagnosed at this age (16). According to Wallace et al. (17), the risk of premature ovarian failure after cancer treatment in these young patients is low (<20%) in case of acute lymphoblastic leukemia and moderate (20%–80%) in case of acute myeloblastic leukemia. A non-negligible percentage of these patients will require bone marrow transplantation or further gonadotoxic treatment for incomplete remission or disease recurrence, and will thus change categories to high risk (>80% risk of premature ovarian failure) (18). It is therefore important to offer fertility preservation options to these patients before cancer treatment. The only option currently available for these often prepubertal patients requiring immediate chemotherapy is ovarian tissue cryopreservation.

Ovarian tissue cryopreservation is usually performed before cancer treatment to avoid the harmful effects of chemotherapy and/or radiotherapy on primordial follicles. Hematologic diseases along with breast cancer represent the most frequent indications for ovarian tissue cryopreservation in most centers (19–22). However, the safety of ovarian tissue autotransplantation in patients with leukemia is a major concern. Indeed, experimental studies by four different groups have demonstrated, by sensitive polymerase chain reaction (PCR) or flow cytometry analysis, that cryopreserved ovarian tissue from patients with leukemia may harbor leukemic cells in >50% of cases and (23–26) subsequently transmit the disease, at least in a xenografting model (24).

For these patients at risk of minimal disseminated disease, follicle culture with in vitro maturation may be a solution (27–32). However, despite advances in this field, viable embryos and live offspring have only been obtained in mice with this technique (33–35), and major hurdles remain before improved culture systems in humans yield competent human oocytes (29). Another approach to restore fertility in these patients is grafting of isolated follicles enzymatically purified from frozen-thawed ovarian tissue. Indeed, ovarian follicles are enclosed in a basement membrane that prevents direct contact between follicular cells on the one hand, and capillaries, white blood cells, and nerve processes on the other (36).

With the objective of grafting isolated preantral follicles (obtained from frozen-thawed ovarian tissue) for the purposes of fertility restoration, procedures were set up by our team to isolate (37), recover (38), and transplant (39, 40) human ovarian follicles. A technique for isolation of human preantral follicles following good manufacturing practice conditions was recently optimized by our team with a view toward clinical application (41).

However, even if isolation of preantral follicles from ovarian cortical fragments of healthy women is now a well-established protocol in our department, for patients with leukemia who are at risk of ovarian involvement, malignant cell contamination of the isolated follicle suspension cannot be excluded. Before these follicles can be grafted to patients with leukemia, it is crucial to make sure that the follicle suspension is free of malignant cells.

As cryopreserved ovarian tissue from leukemia patients is not abundantly available and is therefore precious material, we designed a model of ovarian tissue suspensions from healthy patients artificially contaminated with leukemic cells for this preliminary study. Our study evaluated the safety of our follicle pickup technique and set up a protocol to obtain leukemia cell-free follicle suspensions while maintaining good follicle viability. The sensitivity of the PCR technique and its applicability to follicle suspensions was also investigated in this study.

MATERIALS AND METHODS

Use of human ovarian tissue for this study was approved by the institutional review board of the Université Catholique de Louvain (IRB, 2012, 125). Ovarian biopsies were taken from 10 women (between 22 and 36 years of age) undergoing laparoscopic surgery for benign gynecologic disease, after obtaining their informed consent.

Supplemental Figure 1 (available online) shows the experimental design of the study. Ten fresh human ovarian biopsy samples were enzymatically dissociated. A leukemic cell line (BV-173) was marked with a fluorescent cell tracer (CFDA-SE) and added to the resulting ovarian cell suspension. Follicles were retrieved and divided into two groups: in group 1, follicles were picked up by our usual technique without further treatment; in group 2, follicles were washed three times after pickup. Follicle suspensions in both groups were then investigated for the presence of leukemic cells using fluorescence microscopy as well as PCR. Follicle viability was also analyzed in both groups.

Preparation of Leukemic Cells

The BV-173 cell line was derived from a patient with Philadelphia chromosome-positive acute lymphoblastic leukemia (BCR-ABL b2-a2 fusion gene). This cell line was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ no. ACC 20) and cultured in RPMI 1640 medium + 10% fetal bovine serum (FBS) supplemented with antibiotic solution containing penicillin and streptomycin (GIBCO). One million BV-173 cells were freshly marked at the start of each experiment using the Vybrant CFDA-SE Cell Tracer Kit (V12883; Invitrogen). A preliminary experiment was performed to determine the ideal CFDA-SE concentration to mark the BV-173 cells. Concentrations of 0.5, 1, 2,

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