J Assist Reprod Genet (2014) 31:1567–1568
DOI 10.1007/s10815-014-0357-2

LETTER TO THE EDITOR

No malignancy detected in surplus ovarian tissue from a former Ewing sarcoma patient who experienced relapse four years after being grafted with frozen/thawed ovarian tissue

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Received: 17 September 2014 / Accepted: 23 September 2014 / Published online: 11 October 2014

We have recently published a case report describing the first case of grafting frozen/thawed ovarian tissue for puberty induction to a young girl who had recovered from a malignant disease [1]. She had one of her ovaries frozen at 9 years of age prior to undergoing treatment for Ewing sarcoma, which included pre— and post operative chemotherapy comprising (total accumulated dose per m2), vincristine (21 mg), ifosfamide (60 g), doxorubicin (360 mg), etoposide (2.7 mg), actinomycin (9 mg), and cyclophosphamide (10.5 g) according to the EURO EWING-99 protocol. Subsequently, she underwent gross total surgical resection. Histological examination confirmed resection into healthy tissue. Postoperatively, a total irradiation dose of 41.2 Gy to the tumor bed during 23 treatments was given. When she was 13 years and had no ovarian activity, two of ten pieces of ovarian cortex were grafted to the remaining postmenopausal ovary. The grafted tissue regained activity and stimulated her pubertal development.

In 2013 four and half year after the tissue was grafted, then aged 18 years, the patient experienced a relapse of the original disease with Ewing sarcoma occupying the whole left thoracic cage and metastases in the liver. During the subsequent 7 months, the patient received intensive treatment including vincristine, ifosfamide, etoposide and a limited dose of doxorubicin. The tumor responded well and a macroscopically radical extirpation of the thoracic tumor was done followed by thorax irradiation. The liver metastases regressed in size, but the number was too high to attempt radioablation. The quality-of-life was severely intimidated by the chemotherapy. We therefore refrained from further treatment and the patient died on January 13th 2014.

Although the relapse occurred in the hemithorax away from the site of the remaining ovary, it was important to clarify whether the malignant cells causing relapse could have been introduced via the transplanted ovarian tissue. In order to approach this question the remaining eight pieces of ovarian cortex prepared for transplantation were tested for the possible presence of malignant cells. The parents consented to this plan.

The patient was positive for the *EWS*/*FL1* translocation at first diagnosis and the pathological department's normal PCR based method to detect the *EWS*/*FL1* translocation was used using the *PGK* gene as a house keeping gene. The eight pieces of ovarian tissue were thawed and each piece was divided into six smaller fragments of tissue. Each individual piece was subjected to a manual homogenization and extraction of mRNA. The concentration of mRNA extracted for each individual piece is given in Table 1. The house keeping gene was detected in all samples except for samples 4,1; 4,6; 6,3; 10,1 and all controls were satisfactory. The *EWS*/*FL1* translocation was not detected in any sample. These data indicate that the ovarian tissue did not contain malignant cells. However, since

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Table 1 Concentration of mRNA (ng/μl) in samples extracted for the measurement of the EWS/FLI translocation in eight separate ovarian biopsies each divided into six fragments prior to extraction

Sample/Tube	1	2	3	4	5	6
2	258	316	329	154	436	378
3	522	203	384	319	434	300
4	43	71	279	424	182	40
6	<5	26	10	172	76	11
7	<5	67	<5	7	16	6
8	16	13	<5	<5	<5	<5
9	119	72	143	109	59	138
10	49	111	140	212	124	80

the two originally transplanted ovarian pieces of cortex were not analyzed for the translocation, it cannot with 100 % certainty be excluded.

The most comprehensive analysis of presence of sarcoma cells in ovarian tissue prepared for transplantation performed until now included a total of 16 patients of which nine had Ewing sarcoma [2]. Ovarian tissue was transplanted to immunodeficient mice for 20 weeks and evaluation for the presence of the *EWS*/*FL1* translocation in all cases revealed no signs of malignant cell contamination [2]. However, another study found one case of ovarian tissue positive for the *EWS*/*FL1* translocation out of eight evaluated patients [3]. Recently another study reported on the presence of CD99 positive cells on the surface of ovarian tissue harvested from a Ewing sarcoma patient [4]. Apparently only few studies have specifically looked for ovarian involvement in early stage Ewing sarcoma [5]. The present study suggests that the transplanted ovarian tissue was not involved in the relapse of this patient and the four and a half year period from transplantation to relapse further suggests no ovarian involvement though it cannot be excluded. However, this study does demonstrate that it is very important to closely follow these patients and perform studies to secure the safety of this procedure by detecting potential rare cases of ovarian involvement.

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