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Short Communication

Case report: Stimulation of puberty in a girl with chemo- and radiation therapy induced ovarian failure by transplantation of a small part of her frozen/thawed ovarian tissue

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Abstract ***Aim of the Study:*** To induce puberty by transplantation of frozen/thawed ovarian tissue collected prior to gonadotoxic treatment for a cancer.
Patients and Methods: A 9-year-old girl with Ewing sarcoma had one ovary excised and cryopreserved prior to chemo- and radiotherapy. Functional activity of the remaining ovary was destroyed during treatment. Four and a half years later the girl remained pre-pubertal with postmenopausal levels of FSH. Two of ten pieces of frozen/thawed cortex were transplanted to the remaining ovary in order to stimulate puberty.
Results: Four months after the transplantation FSH returned to low levels. During the following year puberty gradually progressed to Tanner stage B4 and P3 and regular menstrual cycles started. However, after 19 months the function of the graft ceased.
Conclusions: We have shown for the first time in a girl treated for cancer that transplanted ovarian tissue can regain function and secrete estradiol in a sufficient amount to induce puberty. In addition, the majority of her ovarian tissue remains frozen with a possibility to support fertility in adult life.

1. Introduction

Today, many girls and young women survive a cancer disease following aggressive treatment with high-dose chemotherapy and/or radiation.[1] A number of these treatment regimes may as a side-effect eliminate the pool

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of ovarian follicles and render the patient sterile.[2] As a quality of life aspect many patients and/or their parents request preservation of fertility prior to the gonadotoxic treatment.[3] Cryopreservation of ovarian tissue is rapidly gaining ground as a valid method for fertility preservation and is the only option available for pre-pubertal girls.[4–6] Freezing ovarian tissue has an additional advantage, since not only the reproductive potential is stored, but also the functional unit of the ovary, the follicle. Follicles in the transplanted tissue possess the capacity to produce estradiol and other sex hormones that maintain regular menstrual cycles. Sex hormones exert a pleiotropy of different functions in the female body and maintained female steroid producing capacity opens new possibilities. Here we describe how cryopreserved ovarian tissue retrieved from a young pre-pubertal girl with cancer, when transplanted several years later, had the capacity to induce puberty by an endogenous production of estradiol and other sex hormones. This case extends on a recently published case, where cryostored ovarian tissue from a young girl with a benign disease was used for induction of puberty.[7]

2. Case

In February 2005 at the age of 9 years the girl was diagnosed with Ewing sarcoma. Magnetic Resonance Imaging showed a $62 \times 47 \times 42$ mm soft tissue component with bony lesions of the superior pubic ramus. The tumour displaced urethra, vagina and rectum. Biopsy confirmed the diagnosis of Ewing sarcoma. No metastases were detected. After thorough counselling of the parents and the patient, they consented to have one of the two ovaries removed for fertility preservation prior to treatment. The ovary was excised by laparoscopy and transported on ice for 5 h to the laboratory and cryopreserved according to previously published methods.[8,9] Ten pieces of ovarian cortex ($4 \times 5 \times 1$ mm) were frozen in individual vials and stored in liquid nitrogen.

After excision of the ovary, the girl started treatment according to EURO EWING-99 protocol[10,11] with pre- and a post-operative chemotherapy including (total accumulated dose per m^2), vincristine (21 mg), ifosfamide (60 g), doxorubicin (360 mg), etoposide (2.7 mg), actinomycin (9 mg) and cyclophosphamide (10.5 g). Subsequently, she underwent gross total surgical resection. Histological examination confirmed resection in healthy tissue. Post-operatively, a total dose of 41.2 Gy to the tumour bed during 23 treatments was given.

The planned target volume for irradiation therapy included most of the girl's pelvis. She was irradiated with a 3D conformal radiotherapy plan with tangential wedged anterior fields and one posterior wedged field. She suffered severe symptoms of irradiation induced cystitis and pelvic pain and the remaining ovary is likely to have received full irradiation dose.

Four and a half years later (spring 2009) the girl was 13.6-years-old and showed no signs of relapse. She had consistent high levels of FSH (>80 IU/L) and showed no clinical signs of pubertal development. In agreement with the patient and her family, it was decided to transplant two pieces of ovarian cortex to the remaining ovary. Since her levels of gonadotropins were high, follicular development was expected to be stimulated.

The frozen/thawed ovarian cortex was inserted in the remaining ovary through a minilaparotomy. The residual ovary was very small, 0.5 mL in size and was without any sign of ovarian activity. The two pieces of cortex were larger than the ovary itself and an incision through the ovary was prepared in order to accommodate the frozen/thawed cortex with the remaining tissue sutured on to the surface. Thereafter, the ovary was returned to the peritoneal cavity. Her levels of gonadotropins were high and follicular development was expected to be stimulated.

3. Results

After transplantation monthly follow-up was initiated. Four months post-operatively, the level of FSH was reduced from >80 to 9 IU/L and the level of estradiol increased from undetectable to 70 pmol/l. FSH remained in pre-menopausal levels although concentrations on a few occasions exceeded 30 IU/L as seen in Fig. 1. On six occasions after transplantation, levels of AMH and inhibin-B were measured and both remained below the detection limit of the assays in all samples. One year after the transplantation, the patient had menarche, and had regular menstruation 3–4 d with a period of 30 d the next 6 months, after which FSH again increased to postmenopausal level and menstruations ceased. On a few occasions antral follicles with diameters of 5–6 mm were observed by transabdominal ultrasonography confirming ovarian function of the grafts.

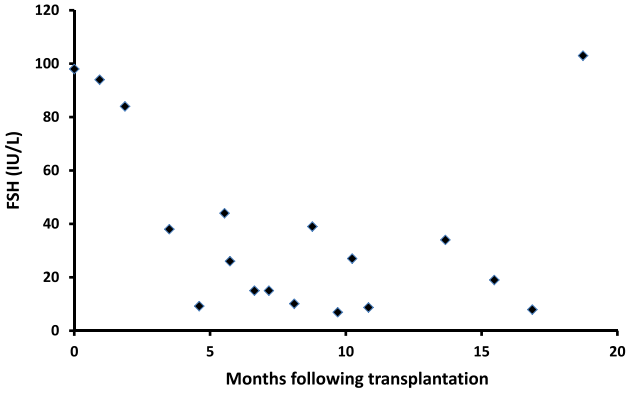

Fig. 1. Levels of FSH (IU/L) following transplantation of frozen/thawed ovarian tissue.