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SHORT COMMUNICATION

Autologous transplantation of cryopreserved ovarian tissue to induce puberty—the endocrinologists' view

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Abstract Transplantation of cryopreserved ovarian tissue has been shown to successfully induce pregnancies. Furthermore, puberty may be induced by transplanted ovarian tissue in girls suffering from premature primary ovarian insufficiency (PPOI) due to gonadotoxic therapy. Therefore, the question arises if ovarian tissue cryopreservation should be recommended for puberty induction in prepubertal girls with cancer prior to gonadotoxic therapies. Although this strategy seems to be more natural than administering exogenous steroid sex hormones, there are some disadvantages from the endocrinological point of view. During physiologic puberty, serum estradiol levels increase very slowly, followed by irregular and finally regular ovulations with progesterone production during the luteal phase. PPOI presents as hypergonadotrophic hypogonadism. When transplanting ovarian tissue in girls with PPOI, the elevated gonadotrophins will promote a sudden follicular growth of one or several follicles with a sharp increase of serum estrogen levels and regular ovulations. This will result into an accelerated pubertal development with the risk of overt weight gain, cutaneous striae and premature growth stop possibly leading to psychological implications.

Conclusion: Transplantation of cryopreserved ovarian tissue should not be recommended as an alternative to medically induced puberty.

What is Known:
- *Ovarian tissue is increasingly cryopreserved before cytotoxic therapies.*
- *Ovarian tissue has been transplanted not only to generate pregnancies but also in induce puberty in children with ovarian failure.*

What is New:
- *Ovarian tissue transplantation in hypergonadotropic adolescents result in a sudden increase of estradiol concentration and an unphysiological fast puberty induction.*
- *Puberty induction by transplanted ovarian tissue appear to be a natural approach but it is not and should therefore not be performed.*

Keywords Ovarian tissue · Cryopreservation · Transplantation · Puberty · Menopause

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Abbreviations

PPOI	Premature primary ovarian insufficiency
FSH	Follicle stimulation hormone
LH	Luteinizing hormone
GnRH	Gonadotropin releasing hormone

Introduction

Cryopreservation of ovarian tissue for fertility preservation prior to cytotoxic therapies is frequently performed in women suffering from cancer. Based on numbers from

the worldwide largest case series in Denmark and from *Ferti*PROTEKT (www.fertiprotekt.com), a fertility preservation network of German speaking countries, it is estimated that several hundreds and in the future even thousands of ovarian tissue cryopreservation are being performed in Europe each year [12]. Ovarian tissue cryopreservation for fertility preservation is not restricted to women in their reproductive years but is also performed in children with cancer, in which this technique is still experimental. However, in children, cryopreserved ovarian tissue might also be used for puberty induction as many of them will develop a chemotherapy induced premature primary ovarian insufficiency (PPOI) that is usually treated by the application of exogenous estrogens. Indeed, successful puberty induction has already been described in two 13-year-old girls with hypergonadotrophic hypogonadism [7, 10]. However, although these reports are of scientific and clinical importance as they prove that follicular growth may be activated in prepubertally cryopreserved ovarian tissue, it also opens the discussion if such a procedure is beneficial for the adolescent or not. Furthermore, the question arises if this therapeutic option should be discussed with the parents and their diseased daughter as an additional advantage of ovarian tissue cryopreservation prior to gonadotoxic therapies.

Ovarian tissue cryopreservation and transplantation to restore fertility—current data

Ovarian tissue cryopreservation is an increasingly performed procedure in women with cancer prior to chemo- and radiotherapy. Each year, about 70 cryopreservations in total are performed in Denmark and within the network *Ferti*PROTEKT, respectively. So far, ovarian tissue has been transplanted 53 times in 41 women in Denmark [9] and 95 times in 74 women within the network *Ferti*PROTEKT [12]. The delivery rate per transplantation was 23 % [12] and the delivery rate per women, including more than one transplantation in some cases, was 32 % [9]. Ovarian tissue activity for at least 1 year was reported in about 75 % of patients in Denmark and in 67 % of patients within the network *Ferti*PROTEKT. The first delivery after transplantation of ovarian tissue that was cryopreserved before menarche suggests that tissue cryopreserved at young age may also be used to generate pregnancies [5].

Safety of ovarian tissue transplantation

Usually, ovarian tissue is cryopreserved in women with cancer prior to chemotherapy and/or radiotherapy. Therefore, ovarian tissue could possibly contain malignant cells bearing the risk of provoking a relapse. So far, several studies have been performed to analyze the risk of malignant cell contamination in cryopreserved ovarian tissue [2]. Furthermore, the risk of ovarian metastasis has been estimated according to cancer type and tumor stage. There were three recurrencies in the Danish case series and one recurrency within the *Ferti*PROTEKT case series all of which did not seem to be related to the transplanted ovarian tissue. In short, according to these data, ovarian tissue transplantation seems to be safe if the tissue is cryopreserved in low risk cases and if tissue biopsies are carefully evaluated by histology, immunohistochemistry, and molecular biology techniques prior to transplantation.

However, data on the risk of ovarian metastasis are still very limited. Furthermore, these data are mainly based on tissue evaluation in adults. As the tumor biology is different in children, the risk of malignant cell contamination in ovarian tissue and the risk of inducing a relapse by tissue transplantation are still unknown.

Endocrinology of transplanted ovarian tissue

So far, the endocrine profile following ovarian tissue transplantation has not been assessed systematically. It seems to resemble the physiologic late pre- and perimenopausal state, respectively, as the ovarian reserve of transplanted tissue is low. The endocrine profile is characterized by fluctuating FSH and estradiol serum levels due to increased FSH levels which are suppressed by sporadic or regular follicular growth with corresponding elevated estrogen production [6]. As transplantation of ovarian tissue has also been suggested to postpone menopause, the advantages and disadvantages from the endocrinological point of view have been critically discussed recently [13]. A major concern, as described in this paper, was the production of estrogen which could not or hardly be adapted to the needs of the individual person and that tissue removal requires surgical intervention.

Endocrinology of natural and medical puberty induction

Physiologic puberty starts with smooth increments of LH and FSH levels stimulated by more frequent and increased GnRH pulses from the hypothalamus. LH and FSH then slowly stimulate the ovaries to produce estrogens that promote the development of secondary sex organs, e.g., the uterus and the mammary gland. Under physiologic conditions, time from breast budding (corresponding to puberty start) to menarche takes about 2.5 to 3 years, while closure of the growth plate occurs about 2–3 years later.

In patients with PPOI, LH and FSH levels rise to an unphysiological high level after puberty induction due to lack of negative feedback control. Therefore, in these patients, very low dose estrogen replacement therapy is initiated as soon as LH and FSH level begin to rise at the age of physiologic puberty. Doses of exogenously administered estrogens

(mostly as pills or transdermal patches) are gradually increased to mimic normal pubertal development. Finally, progestogens are given around the age of normal menarche when the girl is ready for the first menstruation.

Much of the experience of successful puberty induction is derived from girls with 45,X Turner syndrome mostly presenting with streak gonads [3]. In these patients, it has been shown that, first, estrogen replacement therapy should be initiated at the normal age of puberty and should follow the natural path, secondly, postponing puberty will not result in better height outcome, and finally, postponing puberty may negatively affect bone health. In contrast, postponing puberty in patients with Turner syndrome results in lower quality of life. [4]. In patients with Turner syndrome and in patients with a genetic aromatase deficiency (who are not able to synthesize estrogens), it has been shown that ultralow estrogen levels may be needed even before puberty. In a recent study, girls with Turner syndrome being substituted with growth hormone, had a better adult height outcome if treated with ultralow doses of estrogens starting at age 5 [11]. Similarly, in girls with aromatase deficiency, very low doses of estrogens were found to prevent ovarian cyst development [8]. Both studies support the concept of very low dose estrogen supplementation initiated at early age. In contrast, ovarian tissue transplantation would lead to a rapid induction of puberty.

Discussion

The case studies of puberty induction by transplanting ovarian tissue cryopreserved in children before cancer therapy confirm that ovarian tissue can be successfully activated in adolescents resulting in puberty induction. The available safety data also confirm that the risk of transplanting tumor cells is probably low in certain cancer types and disease stages. Tissue transplantation for puberty induction only may not require a laparoscopy as a heterotopic, subcutaneous transplantation can be performed with local anesthesia. A heterotopic site of the transplant may even allow better observation of the transplanted tissue and possibly earlier detection of local recurrencies induced by the transplanted tissue. Furthermore, autologous transplantation of cryopreserved ovarian tissue may allow girls to experience a "natural" puberty. Therefore, this procedure seems to have some advantages and appears to be a simple and low risk treatment to induce puberty (Table 1). However, there are also some disadvantages to consider, which have in part already raised by others [1]:

First, ovarian tissue transplantation cannot prevent and replace hormonal replacement therapy as the tissue survival is limited. Following puberty induction, women will require hormone replacement therapy for many years or even decades. However, in some women, the tissue's activity is less than 1 year [9, 12], requiring more than one transplantation.

Table 1 Advantages and disadvantages of transplantation of ovarian tissue to induce puberty

Advantages
• Cryopreserved tissue can be used for both, generating pregnancies and puberty induction
• No need for regular oral or transdermal medication intake
• Requires only subcutaneous tissue transplantation
• Might improve patients self-confidence as it simulates natural puberty
Disadvantages
• Imposes the risk of transplanting malignant cells
• Individual duration of tissue activity cannot be foreseen and is limited
• Hormone production of transplanted tissue cannot be modified or stopped without surgical tissue removal
• Sudden increase of estrogen concentration resulting in potential side effects as accelerated pubertal development with overt weight gain, cutaneous striae, and premature closure of growth plates

Even though, it can be assumed that tissue cryopreserved at younger age will survive longer, it cannot be expected to survive long enough to completely replace hormone replacement therapy.

Second, the available safety data are too limited to classify this procedure as being safe. In the situation that a girl will need the cryopreserved tissue to become pregnant sometime in the future, it might be acceptable to take a minimal risk as there will not be an alternative. In contrast, for puberty induction, there are well-established medical drug algorithms, which are efficient, safe, and cheap.

Third, even though puberty induction by transplanted tissue appears to be natural, the rapid hormone production due to an imbalanced hypothalamic-pituitary-ovary system is not natural. Physiologic puberty induction is a result of a slow increase of serum estrogen levels stimulated by the hypothalamic-pituitary axis, followed by irregular and later regular ovulations and progesterone secretion.

Fourth, the sudden and overt increase of estrogen production seen a few months after tissue transplantation could induce an accelerated pubertal development leading to side effects. In 50 girls treated for constitutional stature with 7.5–11.25 mg conjugated estrogens per day, side effects such as overt weight gain, headache, nausea, and breast discomfort were noticed [14]. As ovarian tissue transplantation in women with high FSH concentrations lead to an initial activation of a large cohort of follicles, estrogen concentrations similar to those in the study can initially be reached.

For that reason, puberty induction by heterotopically transplanted cryopreserved ovarian tissue should not be judged as a treatment alternative to hormonal replacement therapy. It should not be mentioned as an additional option if tissue cryopreservation is discussed as a fertility preserving technique in girls with cancer prior to gonadotoxic therapies.

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Contributions of the authors M. von Wolff designed the paper and included the technical aspects of ovarian tissue transplantation.
Petra Stute added the gynaecologic endocrinological aspects.
C. Flueck added the pediatric endocrinological aspects.
All authors revised and accepted the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain studies with human participants or animals performed by any of the authors.

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