

M. Huser · E. Jurankova · I. Crha · P. Ventruba ·  
R. Hudecek · J. Zakova · L. Smardova · Z. Kral

## Fertility preservation strategies in women undergoing chemotherapy for haematological malignancy

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**Abstract** As one of the basic modalities of oncological therapy, chemotherapy usually leads to permanent consequences. Infertility is one of the most common consequences resulting from irreversible gonadal damage. The potentially effective method of reproductive function protection in women undergoing chemotherapy for haematological malignancy is administration of GnRH analogues during chemotherapy by creating pre-pubertal hormonal milieu. The other useful methods are the cryopreservation of oocytes and ovarian tissue from patients undergoing anti-cancer therapy. The presented OvarOnko project sets the primary target to verify the potential protective effect of GnRH analogues to protect ovarian tissue over the course of three different chemotherapy regimens in female patients with childbearing potential who suffer from Hodgkin's lymphoma (HL). Another goal of the project is to work out practical conditions and working procedures for the development of the method of the cryopreservation of oocytes and ovarian tissue. The major outcomes of the project will be the verification of efficacy or the lack of efficacy of GnRH analogues in the ovarian function protection and the elaboration of practical conditions and working procedures allowing inclusion of the methods of cryopreservation of oocytes and ovarian tissue in services provided to our patients before anti-cancer treatment. The costs of the two methods of ovarian function protection will be compared. The differences in ovarian response between the patients with HL and non-oncologi-

cal patients recorded in the register of therapeutic cycles of assisted reproduction will be identified using data-mining methods.

**Keywords** Infertility · Chemotherapy · Assisted reproduction · Cryopreservation · GnRH analogues · Hodgkin disease

### Introduction

Pathological processes proceeding in individual organs of the human body can result in damage to the body's reproductive function. Malignant disease and its therapy are major factors that may result in the complete loss of fertility. Remarkable success achieved in the treatment of malignant tumours has intensified efforts not to deprive young patients of the possibility to have children in the future.

### Anticancer therapy and fertility

Tumours that follow cardiovascular diseases are the most frequent cause of death in women and men at reproductive age in spite of enormous advances in oncological diagnostics and therapy. The development of new diagnostic methods allows the detection of tumour at an early stage, and the use of modern methods of chemotherapy and radiotherapy has contributed to the increasing number of patients who have been cured or have shown long-time remission of the disease. Chemotherapy, as one of the basic modalities of anti-cancer treatment, usually leads to permanent negative consequences such as infertility, which is one of the most common consequences resulting from irreversible gonadal damage. Hodgkin's lymphoma (HL) is a typical example of the disease with a good response to chemotherapy and good prognosis *quod vitam* [1].

Depending on the kind of chemotherapy and the number of the administered cycles, gonadal function in female patients with childbearing potential is affected in 70–100%

M. Huser (✉) · E. Jurankova · I. Crha · P. Ventruba ·  
R. Hudecek · J. Zakova  
Department of Gynaecology and Obstetrics,  
University Hospital Brno,  
Obilní trh 11,  
625 00 Brno, Czech Republic  
e-mail: martin.huser@atlas.cz

L. Smardova · Z. Kral  
Department of Internal Medicine and Hematooncology,  
University Hospital Brno,  
Brno, Czech Republic

of cases [2]. The overview of commonly used chemotherapeutic agents affecting fertility is listed in Table 1. The cumulative doses of chemotherapeutic agents resulting in permanent azoospermia in reproductive-age men are shown in Table 2. The negative consequences in male patients can be avoided by the timely collection and cryopreservation of sperm before systematic anti-tumour therapy. One interesting finding is that the results of semen analysis (spermiogram) show differently impaired spermatogenesis in patients with HL even before the initiation of chemotherapy using cytostatic agents. This may indicate a possible connection between sperm quality and the basic disease.

In the case of female patients, the problem of ovarian function protection has not been fully resolved although it concerns not only oncological patients but also women undergoing cytostatic or immunosuppressive therapy for various conditions such as systemic autoimmune diseases, rheumatic diseases, vasculitis, and organ transplant.

### Premature ovarian failure and chemotherapy

Premature ovarian failure is a common long-time consequence of chemotherapy and radiotherapy. From a clinical point of view, it is defined as menstrual cessation that lasts for more than 6 months and occurs in women under the age of 40 years, accompanied with highly elevated levels of gonadotropins [follicle-stimulating hormone (FSH) and luteinizing hormone (LH) above 20 IU/l]. Unlike other rapidly proliferating body tissues, the damage to ovaries is mostly irreversible, as the number of ovarian follicles at birth is limited. The destruction of follicles leads to primary ovarian insufficiency followed by sterility accompanied with symptoms of the menopausal syndrome [3].

The mode of action of chemotherapy in ovaries has not yet been fully explained. However, it is assumed that it produces toxic effects on membrana granulosa cells or oocytes, which ultimately leads to the atresia of a follicle. Alkylating cytostatics interfering in the cell cycle of rapidly proliferating cells affect membrana granulosa cells whose division is controlled by gonadotropins released from the

**Table 1** The overview of gonadotoxic chemotherapeutic agents

Alkylating agents	Cyclophosphamide
	Chlorambucil
	Melphalan
	Busulphan
	Carmustine (BCNU)
	Lomustine (CCNU)
Platina derivatives, Vinca alkaloids, taxans, anti-metabolites	Methoxamin
	Cisplatin, carboplatin
	Vinblastine, Vincristine
	Paclitaxel
	Docetaxel
	Cytosine arabinoside
	Procarbazine

**Table 2** The cumulative dose of chemotherapeutic agents resulting in permanent azoospermia in reproductive age men (Schrader et al. 2001—modified) [24]

Chemotherapeutic agents	Dose (g/m <sup>2</sup> )
Cyclophosphamide	7.5
Procarbacin	2.5
Chlorambucil	1.4
Vincristine	1.0
Paclitaxel	1.3
Chlorambucil	4.0

pituitary gland. Indirect evidence indicates that gonadotropic stimulation in particular is the main prerequisite for the effect of the chemotherapeutic agent on the ovary. The administration of alkylating substances leads to the rapid destruction of mature follicles, the subsequent onset of maturation of immature follicles and the rapid depletion of primordial follicles [4].

The risk of the development of premature ovarian failure in women after chemotherapy depends on the patient's age, type of the gonadotoxic agent and the cumulative dose reached.

### Methods of ovarian function protection

Current modern methods of assisted reproduction (AR) are able to offer to cured oncological patients a chance to have their own children. Over the recent years, efforts have also focused on the development of procedures performed in patients before and during anti-cancer therapy to prevent infertility.

#### 1. Cryopreservation of gametes

The routinely used method, which leads to the preservation of male fertility, is the cryopreservation of sperm performed before anti-cancer therapy [5], which has a long-time tradition at our clinic and is also frequently used by physicians working in most clinical fields. This shows that not only healthcare professionals but also the broad public are acquainted with this method. Oocyte cryopreservation in female patients is an analogy to the above-mentioned method. This method has been elaborated intensively over the last 10 years. However, until now it has not been established in standard practice as a routine method due to a relatively low success rate in achieving fertility from cryopreserved oocytes [6]. Although there has been significant success in this area [7], a number of issues concerning the standardization of the process of cryopreservation and the thawing of human oocytes will have to be addressed.

#### 2. Cryopreservation of embryos

Another frequently used procedure, which can lead to fertility protection before the initiation of anti-cancer therapy, is the performance of the IVF cycle with fertilization and subsequent cryopreservation of em-

bryos after in vitro cultivation. This method has been technically elaborated and introduced in practice. However, its use is limited by a number of serious conditions. The most limiting requirements are the relatively early stage of the oncological process and good health condition of the patient, which allows postponement of the initiation of oncological therapy by ca. 3–4 weeks, and the performance of ovarian stimulation with subsequent retrieval of oocytes [8]. Another limitation, which is legislative and ethical, is the existence of the patient's partner who has to provide his written consent with the performance of the IVF cycle and sperm to fertilise the retrieved oocytes.

### 3. *Cryopreservation of ovarian tissue*

A method of choice in patients who do not meet some of the above-mentioned requirements is the possibility of cryopreservation of ovarian tissue before the start of anti-cancer therapy. The method of retrieval and cryopreservation is currently well-documented [9]. However, the basic limitation of the standardised technique of tissue auto-transplantation after successful anti-cancer treatment and the process of in vitro maturation of immature oocytes in the ovarian tissue obtained in this way. The progress in cryobiology of ovarian tissue obtained with laboratory animals launched a number of ovarian tissue bank projects, which may accelerate further development of this experimental method in standard clinical practice [10]. Recently, the pregnancy and life-birth after ovarian tissue cryopreservation, thaw and auto-transplantation to orthotopic location has been reported in a woman 6 years after successful oncological treatment [11, 12]. However, this new and finally successful fertility preservation option still remains experimental and the efficacy and reliability have not yet been determined. On the other hand, the future of this technique in women with cancer is promising.

### 4. *Pharmacological ovarian protection with GnRH analogues*

All the above-described methods are based on developed AR methods and experiences with cryopreservation of human gametes or embryos. The principal question is whether it is possible to prevent ovarian damage resulting from aggressive chemotherapy. It has been observed repeatedly that girls treated for tumour in pre-pubescent age do not show ovarian damage, as compared with adult females whose germ cells undergo cyclic hormonal stimulation and proliferation [13].

The basic property of oocytes is that they have great germination potential and division capabilities, which pre-determine them to be amongst the first victims of aggressive chemotherapy. On the other hand, the process of folliculogenesis and oocyte maturation is controlled by hormones and affected by a number of endocrine and paracrine substances. Hormones that play a major role in these processes include FSH and LH whose secretion is controlled by the action of gonadoliberin (GnRH). The action of the above-

mentioned hormonal substances allows for stopping oocytes at the level of primordial follicles as it occurs in the pre-pubescent period of a woman's development. The resultant inhibited oocytes will then show significantly lower sensitivity to chemotherapy, which has been confirmed in Israeli pilot studies performed by Blumenfeld et al. [14]. The drug that stops the cyclic secretion of FSH and LH consists of GnRH analogues, which are commonly used in IVF protocols of ovarian stimulation and which are administered continually for a long period of time [15]. The disadvantage of their use is the temporarily increased secretion of gonadotropins for a period of 7–10 days, i.e. flare-up phenomenon (the sensitivity of ovarian tissue to chemotherapy is increased). Another method of choice is the use of GnRH antagonists whose application in combination with a GnRH analogue ensures ovarian suppression within 4–5 days [16]. The major obstacle for the routine use of this protocol is a relatively high price.

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## **Project of reproduction function protection in women undergoing chemotherapy for haematological malignancy (OvarOnko)**

The primary goal of the project of reproductive function protection in women undergoing chemotherapy for haematological malignancy is to verify the protective effect of GnRH analogues administered to protect ovarian tissue in the course of three different chemotherapy regimens in female patients with childbearing potential who suffer from Hodgkin's lymphoma (HL). Another goal of the project is to work out practical conditions and working procedures for the development of the method of the cryopreservation of oocytes and ovarian tissue from patients undergoing anti-cancer therapy.

In the course of the first 2 years of the project, the patients with childbearing potential who were diagnosed with HL will undergo curative anti-cancer therapy. On the basis of staging, the patients will be divided into three groups, depending on the stage of the disease (German Hodgkin Study Group protocol), and then undergo treatment using one of the three chemotherapeutic regimens that are comparable with regard to toxicity and aggressiveness. All patients undergoing chemotherapy will receive GnRH analogues to protect ovarian function by the mechanism of inhibition of folliculogenesis at the pre-pubescent stage. If the initiation of chemotherapy can be postponed in the course of staging and the patient's clinical condition is good, the patient will be offered to undergo ovarian stimulation followed by the cryopreservation of oocytes or embryos (after fertilization using sperm from her partner) or by the cryopreservation of ovarian tissue that will be used in the future. After the successful completion of anti-cancer therapy proceeding according to the established criteria, all patients will be subjected to re-evaluation of the state of the ovarian tissue with regard to

the aggressiveness of the chemotherapy used. The results will be compared with a control set of female patients treated previously without ovarian protection, in whom ovarian function will also be evaluated using the same methodology.

The major outcomes of the project will be the verification of the efficacy or the lack of efficacy of GnRH analogues in the ovarian function protection and the elaboration of practical conditions and working procedures allowing the inclusion of the methods of cryopreservation of oocytes and ovarian tissue in services provided to our patients before anti-cancer treatment. The costs of the two methods of ovarian function protection will be compared. The differences in ovarian response between the patients with HL and non-oncological patients recorded in the register of therapeutic cycles of assisted reproduction (AR) will be identified using data-mining methods.

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## Discussion

The preservation of the function of ovaries depends particularly on the gonadotoxic effect of the required therapeutic protocol and the ovarian reserve of primordial follicles in ovaries, which decreases with age. Hormonal suppression is based on the hypothesis that the ovary in the resting phase is less sensitive to the cytotoxic effects of chemotherapy. This hypothesis was verified both experimentally and in several pilot studies with human ovaries [17, 18]. The activity of the ovary is suppressed after several days. However, the exact mechanism of this protection is not known since primordial follicles that form the decisive part of the follicular reserve are not affected by gonadotropins. The size of the follicular reserve is the key factor for the future ovarian function [19]. Recently Johnson and Tilly has proven on mice that germ cells from bone marrow can continuously replenish the pool of immature follicles, which calls into question the always taught fact that a woman is born with determined count of primordial follicles. One may speculate that GnRH analogues can protect these undifferentiated stem cell lines [20].

Cryopreservation of germ cells is an alternative of pharmacological ovarian protection. It is associated with a number of problems when considered in women before therapy. Because of its structure, the oocyte is very vulnerable during cryopreservation; fertilization and pregnancy occur only sporadically after thawing [21]. Only high quality embryos tolerate the process of freezing and thawing; after successful fertilization of the oocyte with sperm, the procedure has also good pregnancy rates [22].

Great attention has been devoted to the possibility of cryopreservation of ovarian tissue. The collection, preparation and individual protocol of cryopreservation has been thoroughly elaborated in experiments with laboratory animals [9, 10]. The latest reports of a live birth after transplantation of human ovarian tissue [12] has reinforced the clinical potential of ovarian tissue cryopreservation for fertility loss prevention, and there are many scientists

nowadays focusing on how to improve and standardise the efficacy of these processes [23].

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## Conclusion

Clear evidence of the protective effect of GnRH analogues on the ovarian function in the course of chemotherapy and the development of methods of cryopreservation of oocytes and ovarian tissue can be extremely valuable with regard to a number of aspects. The patient can be offered a significantly better quality of life in the case of successful curative therapy of oncological disease. The mentally stressful impact of oncological diagnosis and demanding chemotherapy will be reduced or minimised by another mentally scarring aspect such as infertility. Moreover, the social and economic advantages should not be overlooked when a patient who has undergone costly therapy will be given a chance to start her own family, the basic unit of the development of society.

The protection of the reproductive function requires early and close cooperation of oncologists with a centre specialising in the infertility treatment. The increasing number of women and children who are facing premature ovarian failure and sterility after cancer treatment needs to move all of us to improve current strategies and to develop new strategies of fertility preservation.

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