

# Prevention of ovarian function damage by a GnRH analogue during chemotherapy in Hodgkin lymphoma patients

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**BACKGROUND:** Frequent negative consequence of chemotherapy (CHT) is ovarian damage and premature ovarian failure (POF). Aim of this prospective case–control study is evaluation of GnRH analogue (GnRH-a) administration to patients with Hodgkin lymphoma (HL) during CHT and prevention of ovarian damage depending upon CHT regimen. **METHODS:** Study group consists of 72 patients in fertile age (18–35 years) with HL diagnosis treated in 2004–2005 by curative CHT together with GnRH analogue (Triptorelin) administration according to a standardized protocol. Patients were divided into three groups according to the stage of disease and treated by three types of CHT regimens (A,B,C) with increased cytotoxicity. Ovarian function of all patients was assessed by gonadotrophin levels (FSH, LH) analysis from peripheral blood before treatment and also 6 and 12 month after it. The number of women with POF after CHT in study group was compared with control group ( $n = 45$ , age 18–35 years) of patients treated in 2002–2003 according to the same protocol but without protective GnRH analogue application. **RESULTS:** In study group with GnRH analogue administration during CHT, there was significantly ( $P < 0.001$ ) fewer cases with POF 6 and 12 month after the end of CHT (37.5% and 20.8%, respectively) than in control group (73.3% and 71.1%, respectively). Comparative analysis depending on cytotoxicity of CHT regimen used showed significant differences in percentage of patient with acquired POF between study and control group only in less aggressive CHT protocols. **CONCLUSIONS:** Study showed a significant reduction of ovarian failure risk in women with HL treated with less aggressive CHT regimens plus a GnRH analogue.

**Keywords:** GnRH-agonists; infertility; ovarian failure; Hodgkin lymphoma; chemotherapy

## Introduction

Tumour diseases are the second most frequent cause of death in humans. Despite the rapid development of oncology, earlier interception of the disease and the modern very effective chemotherapeutics, oncological therapy often leaves permanent after-effects. In the Czech Republic, 58 000 women of pre-reproductive or reproductive age contract cancer in a year, which is  $\sim 0.5\%$  of the whole population (Nor, 2006). In 2003, the American National Cancer Institute estimated the number of patients who survived cancer in their childhood in USA as 270 000, i.e. one in a thousand (Jemal *et al.*, 2006). It is estimated that 1 in 250 people will have cancer therapy in their childhood medical history (in 2010) (Bleyer, 1990). According to some epidemiological studies, damage to ovarian function with subsequent sterility and premature ovarian failure (POF) has been observed in 70–80% of cases after successful antitumour therapy (Seli and Tangir, 2005).

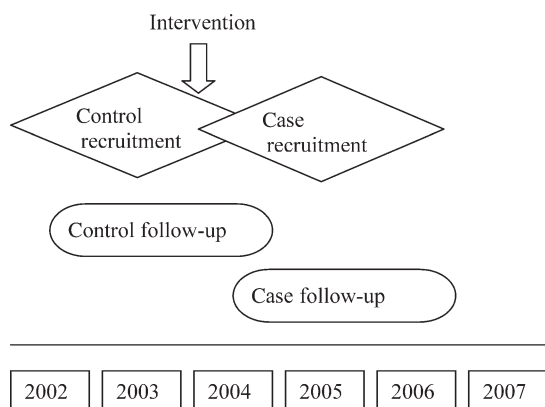
Functional damage to the ovary is due to the destruction of primordial follicles, particularly by the action of some commonly used chemotherapeutics. The exact mechanism of ovarian damage by chemotherapy (CHT) is not yet completely understood. Toxic action of chemotherapeutics on membrana granulosa cells or oocytes leading to rapid follicular atresia is assumed. Cytostatics act in particular on rapidly multiplying cells of the organism, thus also on ovarian cells, especially on those of the membrana granulosa (Sonmezer and Oktay, 2004). Risk of the development of POF depends to a large extent on the patient's age, on the chemotherapeutics and therapeutic regimen used and on the total cumulative dose (Huser *et al.*, 2006). Damage to ovarian functions is caused most frequently by alkylating cytostatics (cyclophosphamide, busulphan, chlorambucil, cytarabine), by vinca alkaloids (vinblastine, vincristine) and the taxans (paclitaxel, docetaxel) (Schrader *et al.*, 2001).

In women, there are several methods of fertility preservation before CHT with heterogeneous outcomes. The most successful are ovarian stimulation and oocyte or embryo freezing and ovarian tissue harvesting and cryopreservation (Oktay, 2006a,b). One of the methods for protecting female reproductive function and for preventing ovarian damage is the administration of GnRH analogues (GnRH-a) during CHT. It is assumed that, due to the administration of GnRH-a, the quiescent (inactive) ovary is less sensitive to the cytotoxic effects of the CHT (Blumenfeld and Eckman, 2005). The protective effect of GnRH-a has been repeatedly demonstrated in animal models (Bokser *et al.*, 1990; Ataya *et al.*, 1995) and also in several human studies (Blumenfeld *et al.*, 1996; Dann *et al.*, 2005; Recchia *et al.*, 2006). These results have been subjected to criticism owing to the heterogeneous patient sets and follow-up protocols, and different regimens and lengths of the CHT used.

Objective of the submitted study (OVARONKO) is the evaluation of the protective effect of GnRH-a administration on ovarian function in patients with Hodgkin's lymphoma (HL) treated with CHT in comparison with a control group, without GnRH-a. This is experimental case-control study with historical controls. Both experimental and control groups were age-comparable and treated by the same CHT protocols. Ovarian functions of control group were evaluated while new patients (Case group receiving GnRH-a) were recruited to the study. Fertility potential of both groups were assessed by standardized methods and then compared. Patient recruitment and follow-up is illustrated on timeline flowchart (Fig. 1). The degree of damage to ovarian function has also been evaluated with respect to the dose and type of the cytotoxic used.

## Materials and Methods

The study was conducted in the years 2004–2007 at the Department of Obstetrics and Gynecology and Department of Internal Medicine and Hematooncology at the Brno University Hospital and Masaryk University School of Medicine, Brno, Czech Republic. The set comprised 72 women with newly diagnosed HL in the period from January 2004 to January 2006. Study has been carried out on Caucasian Czech



**Figure 1:** Study design diagram: timeline flowchart of case (CHT plus GnRH analogue) and control (CHT alone) group recruitment and follow-up

(central European) population. All women included into the study were of fertile age from 18 to 35 years. The next inclusion criteria were presence of both ovaries and absence of ovarian tumours or cysts over 40 mm in diameter demonstrated by vaginal ultrasound examination. Another study inclusion criteria was physiological functioning of the ovaries assessed by determining the FSH and LH levels in peripheral blood taken on the 1st–5th day of the menstrual cycle. Women with LH or FSH values over 15 IU/l were excluded from the study. The cut-off value was established based on definition of ovarian factor of infertility (ASRM, 2006), laboratory definition of POF (Goswami and Conway, 2005) and our experience (Huser *et al.*, 2007a). None of the women included and monitored in the study was on hormonal replacement therapy or hormonal contraception.

All patients underwent the clinical evaluation of the stage of the HL disease according to the standard German Hodgkins Lymphoma Group (GHLG) protocol (Klimm *et al.*, 2005; Draube *et al.*, 2006). On the basis of staging results, the women were distributed into three arms and then treated with three types of chemotherapeutic regimen with increasing cytotoxicity (Table I). Throughout the course of CHT, patients in the experimental (Case) group were administered triptorelin (Diphereline SR 3 mg, Ibsen) in the form of i.m. injections, always once a month and simultaneously with the CHT, in order to inhibit the hormonal functions of the ovaries. The first injection of GnRH-a has been timed to 1st–5th day of the menstrual cycle and the CHT started at least 7 days later to overcome the gonadotrophins flare-up. If the CHT could not be postponed due to risk of HL progression, first dose not triptorelin was given immediately at the end of staging and the flare-up was suppressed by GnRH antagonist cetorelix (Cetrotide 3 mg, Serono) 6 h after triptorelin administration (Mardesic *et al.*, 2004). The administration of GnRH-a ended with the last series of CHT. In addition to GnRH analog co-treatment, if the start of CHT could be postponed during staging, women were given the option of laparoscopic harvesting and ovarian tissue cryopreservation, or ovarian stimulation with cryopreservation of oocytes or embryos (Huser *et al.*, 2007b).

After the completion of oncological therapy, lasting an average 5.3 months, all women included in the study were monitored not only by an oncologist but also by a gynaecologist at intervals of 6 and 12 months after the end of the antitumour therapy. In both visits, the presence or absence of the menstrual cycle was determined, the LH, FSH and estradiol levels were determined always on the 1st–5th day of the menstrual cycle (the sampling was not timed if menstruation was absent), and endometrial thickness and primordial ovarian follicles were examined using vaginal ultrasonography. Normal ovarian function after CHT was determined as the presence of regular menstrual bleeding and on the basis of levels of FSH < 15 IU/l and LH < 15 IU/l in peripheral blood. Where this was not the case, reproductive function was evaluated as POF.

The control set consisted of 45 women of reproductive age (18 to 35 years), who underwent treatment for HL according to the same therapeutic protocol (GHLG) in the years 2002–2004. Control group was recruited partly retrospectively in 2003 and followed-up at the time of recruitment of experimental (Case) group receiving GnRH-a during CHT (see timeline flowchart, Fig. 1). The same inclusion/exclusion criteria were used as in the experimental (Case) group. Vaginal ultrasound examination records were obtained from family doctor providing preventive gynaecological check-ups and women with ovarian tumours or cysts over 40 mm were excluded. Assessment of FSH and LH levels taken on the 1st–5th day of the menstrual cycle was performed during oncological staging, as the recommended test for fertility status according to the GHLG protocol (Draube *et al.*, 2006). Women with LH or FSH values over 15 IU/l were excluded

**Table I.** Type of chemotherapy in relation to result of Hodgkin lymphoma staging (according to German Hodgkins Lymphoma Group protocol—GHLG)

Arm	Description	No. of cycles	Chemotherapeutics
A	ABVD	4	Adriamycin, bleomycin, vinblastin and dacarbazine
B	Combination of ABVD+BEACOPP regimens	4	Adriamycin, bleomycin, vinblastin and dacarbazine+bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine and prednisone
C	BEACOPP regimen	8	Bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine and prednisone

**Table II.** Distribution of female patients included in the study in individual groups and arms

	Group	Total					
		Case	Control				
<b>Chemotherapy type (GHLG protocol)</b>	A	20	27.8%	19	42.2%	39	33.3%
	B	25	34.7%	10	22.3%	35	29.9%
	C	27	37.5%	16	35.5%	43	36.8%
<b>Total</b>		72	100.0%	45	100.0%	117	100.0%

from the study. These women were also followed-up by a gynaecologist 6 and 12 months after completion of CHT, at which points the same data were obtained as for the investigational (Case) group of patients. Use of hormonal replacement therapy or hormonal contraception was avoided during surveillance.

A two-sample binomial test with  $\alpha = 0.05$  was adopted for comparison of groups of patients (Case and Control groups). As several parameters were compared at several time intervals, Bonferroni correction for multiple tests was used for adjusting the level of statistical significance, i.e.  $\alpha = 0.05$  was divided by number of statistical tests to obtain  $\alpha$  for individual test.

## Results

The main division of the patient set into experimental (Case) and control (Control) groups was based on the administration or non-administration of GnRH-a for the protection of ovarian functions. Furthermore, both groups were divided into arms A, B and C according to their respective chemotherapeutic regimen, whereby women were treated with increasing cytotoxicity of the therapy in that order. The number of patients and the percentage representation in individual groups and arms is summarized in Table II. The mean age of patients across the whole set was 30.4 years (median of 30 years). In the experimental group it was 29.0 years, and in the control group it was 32.5 years. There was no significant difference between the two groups with respect to age.

After the general comparison of the Case and the Control groups, fewer cases of POF were found in the group with the administration of GnRH-a during CHT than in the control group. Moreover, the difference in the rate of incidence was statistically significant. Results of the overall comparison of the Case and Control groups at all intervals investigated (before the CHT, 6 and 12 months after CHT) are summarized in Table III. Six months following the termination of therapy, fewer cases of POF according to FSH levels were demonstrated in the group with application of GnRH-a during CHT than in

**Table III.** Results from the overall comparison of the Case and Control groups undergoing chemotherapy (CHT)

	Percentage of women with the given hormone level outside normal (average hormonal levels)	<i>P</i> -value	
			Case % (IU/l) <i>n</i> = 72, age 29.0 years
<b>Status before CHT</b>	<b>FSH</b> 0.0% (3.5)	0.0% (3.3)	1
	<b>LH</b> 0.0% (4.5)	0.0% (4.9)	1
<b>Status six months after CHT</b>	<b>FSH</b> 37.5% (18.1)	73.3% (35.0)	<0.001
	<b>LH</b> 27.8% (16.2)	71.1% (27.9)	<0.001
<b>Status a year after CHT</b>	<b>FSH</b> 20.8% (13.3)	71.1% (31.6)	<0.001
	<b>LH</b> 20.8% (12.3)	66.7% (25.5)	<0.001

the control set (37.5% and 73.3%, respectively;  $P < 0.001$ ). After 12 months, 20.8% cases of POF were found in the Case group in comparison with 71.1% in the Control group ( $P < 0.001$ ).

The protective effect of GnRH-a on ovarian function in both groups of patients (Control and Case) was further evaluated with respect to the degree of toxicity of the chemotherapeutic regimen used. In patients with chemotherapeutic regimen A, the difference between the Case and Control groups in the percentage of women with POF symptoms was statistically significant at both 6 and 12 months after the completion of the CHT, fewer cases of ovarian failure being found in women after the administration of the GnRH-a (Table IV). In patients with chemotherapeutic regimen B, the difference between groups appeared as statistically significant only 12 months after the CHT, and this only in the levels of the FSH hormone (Table V). In chemotherapeutic regimen C, the difference in POF occurrence between the Case and the Control groups was not significant either at 6 or 12 months following the end of CHT (Table VI).

The average ultrasound parameters (endometrial thickness and primordial follicle count on both ovaries) in Case and Control group of patients monitored during gynaecology follow-up 6 and 12 month after CHT are summarized in Table VII. The statistical evaluation of these data was not performed due to incomplete data records and risk of different interpretation by multiple ultrasonographers and ultrasound machines used.

## Discussion

In our experience, the risk of ovarian damage during CHT depends, in particular, on the toxicity and type of the

**Table IV.** Results from the comparison of the Case and Control groups of patients with chemotherapeutic regimen A (as defined in Table I)

CHT—regimen A		Percentage of women with the given hormone level outside normal (average hormonal levels)		<i>P</i> -value
		Case % (IU/l) <i>n</i> = 20, age 30.1	Control % (IU/l) <i>n</i> = 19, age 31.4	
<b>Status before CHT</b>	<b>FSH</b>	0.0% (3.9)	0.0% (3.5)	1
	<b>LH</b>	0.0% (4.0)	0.0% (4.9)	1
<b>Status 6 months after CHT</b>	<b>FSH</b>	5.0% (6.4)	84.2% (38.0)	<b>&lt;0.001</b>
	<b>LH</b>	7.2% (6.2)	84.2% (30.7)	<b>&lt;0.001</b>
<b>Status a year after CHT</b>	<b>FSH</b>	0.0% (4.2)	73.7% (22.4)	<b>&lt;0.001</b>
	<b>LH</b>	0.0% (4.3)	57.9% (20.6)	<b>&lt;0.001</b>

**Table V.** Results from the comparison of the Case and Control groups of patients with chemotherapeutic regimen B (as defined in Table I)

CHT—regimen B		Percentage of women with the given hormone level outside normal (average hormonal levels)		<i>P</i> -value
		Case % (IU/l) <i>n</i> = 25, age 29.7	Control % (IU/l) <i>n</i> = 10, age 33.2	
<b>Status before CHT</b>	<b>FSH</b>	0.0% (3.3)	0.0% (3.1)	1
	<b>LH</b>	0.0% (4.1)	0.0% (5.0)	1
<b>Status 6 months after CHT</b>	<b>FSH</b>	24.0% (10.0)	70.0% (28.0)	0.016
	<b>LH</b>	20.0% (12.8)	60.0% (26.4)	0.028
<b>Status a year after CHT</b>	<b>FSH</b>	8.0% (7.8)	70.0% (37.2)	<b>&lt;0.001</b>
	<b>LH</b>	24.0% (11.2)	70.0% (29.5)	0.016

chemotherapeutic regimen used, and on the reserve of primordial follicles, which is determined in particular by the age of the patient.

Most probably, the crucial factor for success is the size of the ovarian reserve of primordial follicles (Huser *et al.*, 2007b). The so far best known laboratory marker of ovarian reserve expressing the function of the ovary is basal FSH assessment in peripheral blood (Meskhi and Seif, 2006). There are also new laboratory markers of ovarian reserve, e.g. inhibins (INH A, B) and anti-Mullerian hormone or assessment by transvaginal ultrasonography (antral follicle count or volume). These new markers of ovarian reserve are either more costly or not routinely available and are not considered better or markedly more precise than the gold standard represented by basal FSH (van Rooij *et al.*, 2005). Ovarian reserve can also be influenced by major pathologies of the ovary—suspicious ovarian masses, endometriosis, dermoidal cysts, inborn errors, etc. On the other hand, small ovarian follicular cysts do not influence ovarian function.

Women's age plays important role in the risk of POF after CHT usually explained by follicle 'numerus fixus' hypothesis taught for many decades that the number of primordial follicles in a woman's ovary is predetermined and falls over the duration of her lifetime. There was no significant difference between the Case and the Control groups with respect to women's age. Within age limit chosen in the study (18–35 years), the differences in ovarian reserve should not have major impact on the

**Table VI.** Results from the comparison of the Case and Control groups of patients with chemotherapeutic regimen C (as defined in Table I)

CHT—regimen C		Percentage of women with the given hormone level outside normal (average hormonal levels)		<i>P</i> -value
		Case % (IU/l) <i>n</i> = 27, age 30.6	Control % (IU/l) <i>n</i> = 16, age 31.4	
<b>Status before CHT</b>	<b>FSH</b>	0.0% (3.3)	0.0% (3.2)	1
	<b>LH</b>	0.0% (5.2)	0.0% (5.0)	1
<b>Status 6 months after CHT</b>	<b>FSH</b>	74.1% (34.2)	62.5% (35.8)	0.428
	<b>LH</b>	51.9% (26.7)	62.5% (25.4)	0.503
<b>Status a year after CHT</b>	<b>FSH</b>	48.1% (25.1)	68.8% (39.0)	0.194
	<b>LH</b>	33.3% (19.3)	75.0% (28.8)	0.012

**Table VII.** Ultrasound findings before and after CHT in Case and Control group

Average ultrasound parameters		Case group <i>n</i> = 72, age 29.0 years	Control group <i>n</i> = 45, age 32.5 years
<b>Status before CHT</b>	<b>Endometrial thickness (mm)</b>	4.8	-
	<b>Follicle count (<i>n</i>)*</b>	7.5	-
<b>Status six months after CHT</b>	<b>Endometrial thickness (mm)</b>	2.3	2.1
	<b>Follicle count (<i>n</i>)*</b>	4.2	5.3
<b>Status a year after CHT</b>	<b>Endometrial thickness (mm)</b>	4.2	3.8
	<b>Follicle count (<i>n</i>)*</b>	6.4	4.6

\* - number of primordial follicles (2–4 mm in diameter) in both ovaries.

results. The age differences between the arms according to CHT type have not been calculated mainly due to low number of patients within arms. This fact could represent the confounding factor and explain different result in different types of CHT regimen.

Another positive effect of the GnRH-a administration during CHT is a marked reduction in the intensity and frequency of menstrual bleeding or irregular bleeding from the genital tract, often in pancytopenic women, with the subsequent necessity for blood transfusion. As the cost of supportive therapy with products containing GnRH-a is several times lower than hemotherapy, significant economic savings can be made.

The presented study was performed on 72 patients in the experimental group and 45 controls. The size of case group is quite large and homogenous in comparison with already published studies (Blumenfeld, 2002; Blumenfeld *et al.*, 2002). The controls were historically selected, but followed-up by gynaecologist and hormonally during recruitment of experimental group. The authors were not able to recruit more controls with complete medical records fulfilling the same inclusion/exclusion criteria as the experimental (Case) group, especially presence of basal FSH and LH levels. After consultations with statisticians, the size of control group was finally resolved as sufficient, if proper and sufficient statistical tools were used (as described in Materials and Methods section).

The exact mechanism of protective effect of GnRH-a of ovarian function is unclear. It is assumed that the mechanism of action is based on the fact that the hormone-inactivated ovary is less sensitive to the cytotoxic effect of the CHT. The GnRH-a inhibition of the hypothalamo-pituitary axis creates a temporary 'pre-pubertal' hormonal environment in the body of a woman of reproductive age during the CHT. Nevertheless, the exact mechanism of this protective effect of GnRH-a in humans at the molecular level is not entirely clear (Blumenfeld, 2002; Blumenfeld *et al.*, 2002). There is, according to Meirow *et al.* (2004), a physiological FSH increase during CHT followed by subsequent increase in the number of maturing primordial follicles. The administration of GnRH-a disrupts the FSH increase and thus fewer primordial follicles are destroyed by the action of chemotherapeutics (Meirow *et al.*, 2004). Another theory emphasizes the decrease *in utero* ovarian perfusion after the administration of GnRH-a, and the resulting lower sensitivity of the ovary to CHT (Falcone and Bedaiwy, 2005). According to the papers by Meduri *et al.* (2003), GnRH receptors were found on the granulosa cells of primordial follicles; the activation of these receptors prevents cellular apoptosis. Other anti-apoptotic molecules acting on a different population of ovarian cells are also being investigated, e.g. the sphingosine-1-phosphate (S-1-P) (Hancke *et al.*, 2007).

Very interesting observations have been published by Johnson *et al.* (2005): they demonstrate the development of new primordial follicles from specific bone marrow stem cells in a mouse model (stage-specific embryonic antigen 1) and their nidation in the ovary. One might speculate that the GnRH-a used during CHT have a favourable effect on the development of new primordial follicles. This theory would explain sporadic cases where a healthy child is born to a woman with clearly demonstrated ovarian failure following CHT, often many years after undergoing antitumour therapy. In 2006, a case report was published describing the spontaneous conception and birth of a healthy child to a woman with lost ovarian function after CHT, several months after auto-transplantation of ovarian tissue into the forearm subcutis (Oktay, 2006a,b). The insertion of healthy frozen-thawed ovarian tissue into the body could activate oocyte production in the ovary by a still unknown mechanism.

It should be realized that the suppression of ovarian function with GnRH-a only starts 7–10 days after administration. During this short time interval, folliculogenesis is temporarily activated owing to the flare-up phenomenon, and therefore the sensitivity of the ovary to the cytotoxic effect of the CHT is temporarily increased (Mordel and Schenker, 1993). Therefore, the administration of GnRH-a must start sufficiently early, preferably ~10 days before the start of CHT. The rapid onset of the suppression of the hypothalamo-pituitary axis due to the administration of GnRH-a also depends on the menstrual cycle phase (approximately within 10 days from the first day of menstruation), and it is fastest in the early follicular phase. In order to suppress the undesirable flare-up phenomenon after the GnRH-a administration, semi-depot GnRH antagonists (cetorelix, Cetrotide 3 mg, Merck-Serono) may also be used within several hours after the GnRH-a administration (Mardesic *et al.*, 2004). The only

problem of GnRH antagonist use in practice is their high price in comparison with GnRH agonists and the need of administration much more frequently—the semi-depot cetorelix lasts in blood only 4–5 days. The planning and timing of the supportive therapy with GnRH-a in cooperation with an oncologist is thus very important as early as the first day of diagnosis of cancer. The comprehensive protection of the reproductive functions of fertile women requires early and close cooperation of the oncological centre with the department of reproductive medicine. In our practice, this cooperation could be ensured by the creation of the interdisciplinary Fertility Protection Centre at the Brno University Hospital and Masaryk University School of Medicine.

The presented OVARONKO study demonstrated the positive effect of the administration of GnRH-a on ovarian functions during CHT, and a statistically significant reduction in POF risk due to the administration of chemotherapeutics in women treated for HL. In the overall comparison, there were fewer cases of POF in the group with GnRH-a administration during the CHT than in the control group, both 6 and 12 months after the end of CHT. However, the protective effect of GnRH-a on ovarian function is weak, if high-dose and combination chemotherapeutic regimens must be used in advanced stages of the HL. The result was confirmed on cancer patients by case–control study, whose design is ethically acceptable. Randomized controlled trial would be definitely helpful to further support our findings. The mechanism of protective effect of GnRH agonists on human ovary is still unknown and further investigation is needed.

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