GYNECOLOGICAL ENDOCRINOLOGY
2021, VOL. 37, NO. 1, 46–50
https://doi.org/10.1080/09513590.2020.1753033

Taylor & Francis
Taylor & Francis Group

PREMATURE OVARIAN FAILURE

An assessment of the protective effect of gonadotropin-releasing hormone agonist and antagonist on bleomycin-induced ovarian toxicity in rats

Tolga Atakul[a], Ahter Tanay Tayyar[b], Özgür Deniz Turan[a], Serkan Yaşar Çelik[c], Mustafa Yılmaz[d], Mert Küçük[e], Hasan Yüksel[a] and Buket Demirci[f]

[a]Department of Obstetrics and Gynecology, Faculty of Medicine, Adnan Menderes University, Aydın, Turkey; [b]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Faculty of Medicine, Bahcesehir University, İstanbul, Turkey; [c]Department of Medical Pathology, Faculty of Medicine, Sıtkı Koçman University, Muğla, Turkey; [d]Department of Medical Biochemistry, Faculty of Medicine, Adnan Menderes University, Aydın, Turkey; [e]Department of Obstetrics and Gynecology, Faculty of Medicine, Sıtkı Koçman University, Muğla, Turkey; [f]Department of Medical Pharmacology, Faculty of Medicine, Adnan Menderes University, Aydın, Turkey

ABSTRACT

The aim of this study is to evaluate the effect of GnRH agonist or GnRH antagonist therapy on bleomycin-administered rats by examining ovarian follicle counts and AMH levels. A total of 30 female Wistar albino rats aged 4–6 months were randomly divided into 4 groups. First, an intramuscular injection of bleomycin (30 mg/m^2) was administered to all except the control group on the 1st, 8th and 15th days. The control group (Group I) was administered 0.1 mL intramuscular saline on those days. The bleomycin group (Group II) was followed up without any further treatment. The bleomycin + GnRH agonist group (Group III) was administered subcutaneous GnRH agonist triptorelin (1 mg/kg) at the same time as the bleomycin injections. The bleomycin + GnRH antagonist group (Group IV) was administered 1 mg/kg cetrorelix acetate subcutaneously, concurrently with the bleomycin. Although AMH levels were lower in the bleomycin group than in all the other groups, there was no statistically significant difference between the groups in terms of AMH levels ($p > .05$). In the bleomycin + cetrorelix acetate and bleomycin + triptorelin groups, significantly higher primordial, secondary and tertiary follicle counts were determined compared to the bleomycin group ($p < .001$). In conclusion the harmful effects of bleomycin on ovarian reserve can be reduced by the simultaneous administration of GnRH agonist or GnRH antagonist.

ARTICLE HISTORY
Received 9 November 2019
Revised 12 March 2020
Accepted 4 April 2020
Published online 14 April 2020

KEYWORDS
Bleomycin; ovarian reserve; GnRH agonist; GnRH antagonist; AMH

Introduction

Chemotherapy-induced premature ovarian failure (POF) is a serious health problem with significant long-term effects, occurring after chemotherapy in premenopausal women. POF leads to negative reproductive outcomes, such as infertility, but may also lead to non-reproductive negative consequences, such as osteoporosis and early cardiovascular disease [1,2]. With an increasing number of patients undergoing cancer treatment in recent years, knowledge of and experience with the late effects of cancer treatments have also increased.

Malignant germ-cell ovarian tumors are usually seen in females of reproductive age. Depending on the age of the patient and the extent of the disease, organ-preserving surgery followed by chemotherapy may be applied [3]. The BEP (Bleomycin + Etoposide + Cisplatin) protocol is the most preferred chemotherapy for malignant germ-cell ovarian tumors that has been shown to be effective and reliable in recent years [3]. Gonadotoxic chemotherapy often results in premature ovarian failure and infertility. The toxic effects of cisplatin and etoposide chemotherapeutic agents on the ovaries have been shown in many studies, but the harmful effects of bleomycin on ovaries have not yet been fully clarified [4–6].

Serum AMH is a very sensitive indicator of follicular depletion and recovery in women during and after chemotherapy. Furthermore, it allows for the detection of differences in ovarian toxicity between chemotherapy regimens [7,8].

It has been shown that GnRH (gonadotropin-releasing hormone) agonist and antagonist agents can be used to reduce the harmful effects of chemotherapy on ovarian reserve [9,10]. Despite, GnRH agonists has entered clinical use and it is now recommended by some of major guidelines, the mechanism of action of GnRHa to induce ovarian suppression is not still clearly identified [11–14].

Similarly, this study aimed to histopathologically and biochemically reveal the effect of single-dose GnRH agonist and antagonist in the reduction of the negative effect of bleomycin on rat ovaries.

Materials and methods

This controlled experimental study was carried out in the Adnan Menderes University Faculty of Medicine animal research unit. The study was conducted in accordance with international ethical rules and with the approval of Adnan Menderes University Faculty of Medicine Animal Experiment Ethics Committee.

CONTACT Ahter Tanay Tayyar drahtertayyar@yahoo.com Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Faculty of Medicine, Bahcesehir University, İstanbul, Turkey

Animals

A total of 30 female Wistar albino rats, each weighing 220–270 g, were used. The room temperature was maintained at 24 °C ± 2 °C, and a 12-h light-dark cycle and adequate ventilation were provided. The animals had unlimited access to food and water. This study was conducted in accordance with international guidelines on the ethical use of animals (HADYEK 64583101/2015/135).

Drugs

A total of 30 rats with normal menstrual cycles were randomly divided into 4 groups. Intramuscular saline only (0.1 mL) was administered to the control group (Group I, $n = 8$). The other 3 groups received 30 mg/m2 intramuscular bleomycin (Bleomycin sulfate, Bleocin-S® 15 mg/flacon, Onko Ilac San ve Tic A.S., Istanbul, Turkey) on days 1, 8 and 15 according to the standard BEP protocol [15]. The bleomycin group (Group II, $n = 7$) was followed up without any further treatment after bleomycin administration. The bleomycin + GnRH agonist group (Group III, $n = 7$) was administered triptorelin acetate (Decapeptyl® Depot 3.75 mg, Ferring, Istanbul, Turkey) subcutaneously at a dose of 1 mg/kg with every administration of bleomycin. The bleomycin + GnRH antagonist group (Group IV, $n = 8$) was administered cetrorelix (Cetrorelix acetate, Cetrotide® 0.25 mg, Merck Serono, Istanbul, Turkey) subcutaneously at the dose of 1 mg/kg concurrently with each administration of bleomycin [9]. For measurement of the body surface area of the rats, the table described by Gouma et al. was used [16].

Intraperitoneal administration of drugs was avoided to prevent direct toxic and sclerosing effects on the ovaries. Thus, the evaluation of the effect of treatment on the parenteral route was provided. After administration of the drugs, one regular cycle schedule was planned for bleomycin half-life and antimetabolic effect. After the 1-month follow-up of the rats, bilateral oophorectomy was performed. Intracardiac blood samples were taken, and the animals were then sacrificed by cervical dislocation under xylazine and ketamine anesthesia.

Anti-Müllerian hormone measurement

The serum level of anti-Müllerian hormone (AMH) was determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Rat ELISA kit, 201-11-1246, Baoshan District, Shanghai, China). The rat AMH ELISA kit sensitivity was 0.101 ng/mL with a coefficient of variation (CV) of <5%. Procedures were performed according to the manufacturer's instructions.

Histopathological evaluation and follicle counting

The rat ovaries were bilaterally removed and left overnight in buffered 10% formaldehyde solution. After fixation in paraffin blocks, serial sections were prepared in 5 µm slices and five random samples were taken from each ovary to assess follicular activity. Following deparaffinization and rehydration, the sections were stained with hematoxylin and eosin (HE).

Follicles containing oocytes were classified according to developmental stages by two experienced histopathologists; each section divided 12 areas and the antral follicles were then counted in all areas of. Follicle counting and classification were performed according to the criteria defined by Myers et al. [17]. Follicle classification is divided into 4 groups—primordial, primary, secondary and tertiary (mature) follicles. In a primordial follicle, oocyte is, surrounded by spindle-shaped single-row granulosa cells. It is characterized by the appearance of a flattened granulosa layer that envelops all or part of the oocyte. In the primary follicle, the oocyte periphery is covered with single rows of cuboidal granulosa cells. The secondary follicle is characterized by a layer of multiple cuboidal granulosa and the presence of zona pellucida. It is also called the preantral follicle. In the tertiary (antral) follicle, follicle fluid accumulation and cumulus oophorus formation are observed in the intercellular space of the granulosa cells. In this phase, an oocyte-adjacent single space and a large, antral space can be selected.

Statistical analysis

The SPSS 22.0 (IBM Corporation, Armonk, New York, USA) program was used to analyze the variables. The normal

Table 1. Results of the evaluations of AMH levels and follicles in all groups.

	Anti-mullerian hormone (AMH) Mean (SD)	Primordial follicle Mean (SD)	Primary follicle Mean (SD)	Secondary follicle Mean (SD)	Tertiary follicle Mean (SD)
Control ($n = 8$)					
I	4.39 (1.34)	70 (9.53)	62.63 (8.50)	52.87 (6.58)	21.12 (2.99)
Bleomycin ($n = 7$)					
II	3.56 (1.21)	49.42 (4.58)	42.86 (6.98)	37.71 (5.94)	10.43 (2.51)
Bleomycin-cetrorelix ($n = 7$)					
III	4.08 (0.77)	59.14 (3.18)	47 (4.35)	46.86 (5.34)	15.28 (2.14)
Bleomycin-Triptorelin ($n = 8$)					
IV	3.61 (0.92)	57.75 (3.73)	47.5 (6.09)	45.5 (4.03)	15.75 (2.25)
p Value (General)	0.406	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Pairwise comparison					
I→II	ns	**<0.001**	**<0.001**	**<0.001**	**<0.001**
I→III	ns	**0.002**	**<0.001**	**0.046**	**<0.001**
I→IV	ns	**0.001**	**<0.001**	**0.013**	**<0.001**
II→III	ns	**0.007**	0.259	**0.005**	**0.001**
II→IV	ns	**0.016**	0.193	**0.012**	**<0.001**
III→IV	ns	0.671	0.887	0.654	0.723

OneWay: ANOVA; Post Hoc Test: Fisher's Least Significant Difference (LSD); SD:Standard deviation; ns:not significant; AMH: Anti Mullerian Hormone.

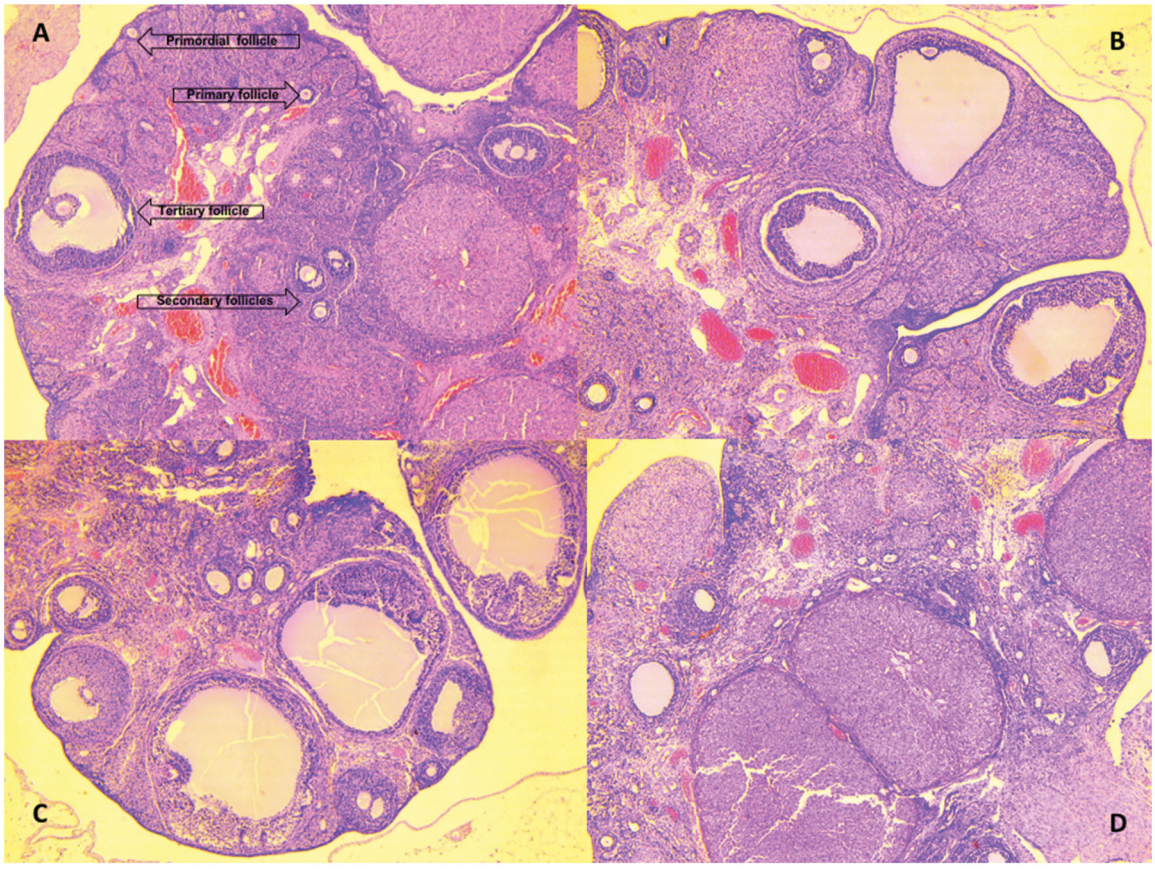

Figure 1. Photograph of study groups with hematoxylin-eosin staining. (A) Control; (B) Bleomycin; (C) Bleomycin + GnRH agonist; (D) Bleomycin + GnRH antagonist.

distribution weight of the data was evaluated with Levene's test of variance homogeneity with the Shapiro-Wilk test. The one-way ANOVA test was used to compare more than one group according to quantitative variables, and the Fisher's least significant difference (LSD) and Games-Howell tests were used for binary comparisons. Quantitative variables were stated as mean ± standard deviation (SD) and categorical variables were shown as number (n) and percentage (%). Variables were examined at a 95% confidence interval, and a value of $p < .05$ was considered statistically significant.

Results

Assessment of plasma AMH levels

The AMH level in the control group was found to be slightly higher than those of the other groups ($p = .406$). In addition, there was no statistically significant difference between the groups in terms of AMH levels ($p > .05$) (Table 1).

Evaluation of ovarian follicle count

The number of follicles in the control group was higher in all series ($p < .001$). A significant decrease in the bleomycin group was observed in the follicle series, especially in the primordial, secondary, and tertiary series ($p < .001$). When the bleomycin and the bleomycin + GnRH agonist groups were compared, there was a significant difference between the primordial, secondary and tertiary follicle counts ($p < .001$). Similar results were also observed in the bleomycin + GnRH antagonist group ($p < .001$) (Table 1, Figure 1).

Discussion

Infertility is a health problem that affects younger cancer patients, particularly patients who are diagnosed with germ-cell tumors in the third decade of life, then undergo chemotherapy while still at an age of fertility. Even after advanced germ-cell ovarian tumors, chemotherapy has been attempted following fertilization-preserving surgery [18]. Therefore, the evaluation of the safety of chemotherapy in terms of its effects on fertility is an important element the ensuring the long-term survival of patients after surgery.

In all cases of yolk-sac tumors, BEP administration is the standard treatment after primary surgery. Many guidelines support the BEP protocol in the treatment of yolk-sac tumors. [19]. In a study of the effect of the BEP protocol on menstrual cycle and ovarian function, no ovarian reserve deterioration, even in cumulative BEP regimens, was shown [20]. The toxic effects of cisplatin and etoposide on germ cells are known to be long lasting. However, there have not been sufficient studies on the effect of bleomycin on ovarian reserve. Therefore, the aim of this study was to determine the effect of bleomycin therapy on ovarian preservation and to evaluate this effect with GnRH agonist and GnRH antagonist administration.

In this study, the administration of bleomycin alone on the days appropriate to the BEP regimen was seen to result in a significant reduction in all follicular series. The same effect was not detected for AMH levels. The significant reduction in primordial follicles was noteworthy, regardless of AMH levels.

In a multi-center retrospective study conducted with 211 females with yolk-sac tumors, the prognostic and long-term fertility effects of postoperative BEP and non-BEP chemotherapy were evaluated. The BEP regimen was found to be superior to

non-BEP protocols in terms of 5 year survival ($p = .0009$). In addition, 70% of nulliparity patients who received BEP treatment were observed to develop a healthy pregnancy after treatment. These results were found to be similar to the non-BEP protocol. Therefore, it has been emphasized that BEP treatment is the most reliable treatment regimen in recent years [19].

For young cancer patients who have recently received chemotherapy with an increased survival after germ-cell tumor treatment, it is important to be able to predict the long-term toxic effects of chemotherapy on fertility. The results of the German Hodgkin Study Group (GHSG) randomized controlled phase-two trial (Protection of Ovarian Fertility), undertaken to evaluate the protective effect of GnRHa and OCS (oral contraceptive) ovaries in female patients with advanced HL, showed that BEP regimens were ineffective. Therefore, it was indicated that alternative prophylactic methods for fertility prior to chemotherapy may be appropriate [21].

As noted in many studies, the severity of ovarian failure is closely related to the class of drug used in treatment, the treatment dose and the age of the patient [22]. Many growth factors, as well as some hormones and cytokines, are responsible for the conversion of primordial follicles to primary follicles. In order to evaluate the persistent effect of chemotherapy protocols on the early reproductive ability of women, it is necessary to fully explain the mechanism of primordial follicle involvement.

Increased preantral follicle loss in women leads to early reproductive loss. Bleomycin also induces an increase in free-oxygen radicals, oxidatively degrading the DNA strand in tumor cells and inducing apoptosis. Bleomycin is therefore responsible for cell apoptosis, especially in primordial follicles [23].

There are studies showing the protective efficacy of GnRH agonist and oral contraceptives using concomitant chemotherapy to protect fertility [24,25]. In recent meta-analysis Lambertini et al. finds ovarian suppression with GnRHa during chemotherapy as an available option to reduce the likelihood of chemotherapy-induced POF in premenopausal patients with early breast cancer [26]. The randomized study by Gilani et al. some patients received BEP protocol for ovarian cancer treatment and they showed that GnRHa has a potential protective effect on ovarian function [27]. On the other hand there are studies revealed that there was no impact of GnRH agonists on the prevention on ovarian function [28,29]. Addition of GnRH antagonist to chemotherapy is another treatment option to POF. It is documented that GnRH antagonist reduces chemotherapy induced ovarian follicular destruction in several studies [30,31].

These treatments are thought to reduce the serum gonadotropin levels in the patients, to inhibit follicle development in the ovaries and also decreases vascularity, thereby reducing the concentration of the chemotherapeutic agents to protect the ovaries during chemotherapy [10,32].

Studies conducted in recent years have shown that the AMH level can be used as a highly sensitive tool for predicting ovarian reserves [33,34]. However, there are also studies that do not detect a relationship between ovarian reserve biomarkers and infertility [35]. Using the AMH level to predict folliculogenesis in the ovaries has been shown to be more sensitive than using the inhibin B, estradiol, basal (3^{rd} day) FSH and LH levels for the same purpose [36]. Interestingly, after chemotherapy, AMH levels change rapidly and persistently for a period of time. This may explain how the toxic effect is more likely to affect primordial and preantral follicles. The co-evaluation of AMH and follicle counts increases sensitivity and predictive clinical benefit [37].

To the best of our knowledge, this is the first study to evaluate the harmful effect of the application of bleomycin alone on ovarian reserve and the use of GnRH agonist and GnRH antagonist to reduce this effect. In conclusion, bleomycin administration can be considered to cause a significant decrease in ovarian follicle counts, and this effect can be reduced by concurrent GnRH agonist or GnRH antagonist administration. The same effect was not detected for AMH levels. Perhaps large scale studies or more sensitive AMH kits will be able to detect this. Further research is needed in this regard.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Ahter Tanay Tayyar http://orcid.org/0000-0001-9491-9998

References

[1] Gordon AM, Hurwitz S, Shapiro CL, et al. Premature ovarian failure and body composition changes with adjuvant chemotherapy for breast cancer. Menopause. 2011;18(11):1244–1248.
[2] van Leeuwen FE, Ng AK. Long-term risk of second malignancy and cardiovascular disease after Hodgkin lymphoma treatment. Hematology Am Soc Hematol Educ Program. 2016; 2016(1):323–330.
[3] de La Motte Rouge T, Pautier P, Rey A, et al. Prognostic factors in women treated for ovarian yolk sac tumour: a retrospective analysis of 84 cases. Eur J Cancer. 2011;47(2):175–182.
[4] Meissner J, Tichy D, Katzke V, et al. Long-term ovarian function in women treated with CHOP or CHOP plus etoposide for aggressive lymphoma. Ann Oncol. 2015;26(8):1771–1776.
[5] Bedoschi G, Navarro PA, Oktay K. Chemotherapy-induced damage to ovary: mechanisms and clinical impact. Future Oncol. 2016;12(20): 2333–2344. Oct
[6] Morgan S, Lopes F, Gourley C, et al. Cisplatin and doxorubicin induce distinct mechanisms of ovarian follicle loss; imatinib provides selective protection only against cisplatin. PLoS One. 2013;8(7): e70117.
[7] Peigné M, Decanter C. Serum AMH level as a marker of acute and long-term effects of chemotherapy on the ovarian follicular content: a systematic review. Reprod Biol Endocrinol. 2014;12(1):26.
[8] Morarji K, McArdle O, Hui K, et al. Ovarian function after chemotherapy in young breast cancer survivors. Curr Oncol. 2017;24(6): e494–e502. Dec
[9] Li X, Kang X, Deng Q, et al. Combination of a GnRH agonist with an antagonist prevents flare-up effects and protects primordial ovarian follicles in the rat ovary from cisplatin-induced toxicity: a controlled experimental animal study. Reprod Biol Endocrinol. 2013; 11(1):16.
[10] Fisch B, Abir R. Female fertility preservation: past, present and future. Reproduction. 2018;156(1):F11–F27. Epub 2018 Mar 26.
[11] Paluch-Shimon S, Pagani O, Partridge AH, et al. ESO-ESMO 3rd international consensus guidelines for breast cancer in young women (BCY3). Breast. 2017;35:203–217. Oct
[12] Oktay K, Harvey BE, Loren AW. Fertility preservation in patients with cancer: ASCO clinical practice guideline update summary. J Oncol Pract. 2018;14(6):381–385. Jun
[13] Lambertini M, Horicks F, Del Mastro L, et al. Ovarian protection with gonadotropin-releasing hormone agonists during chemotherapy in cancer patients: from biological evidence to clinical application. Cancer Treat Rev. 2019;72:65–77. Jan
[14] Poggio F, Lambertini M, Bighin C, et al. Potential mechanisms of ovarian protection with gonadotropin-releasing hormone agonist in breast cancer patients: a review. Clin Med Insights Reprod Health. 2019;13:117955811986458. 31
[15] Azrif M, Leong YK, Aslan NM, et al. Bleomycin, etoposide and cisplatinum (BEP) chemotherapy for metastatic germ cell tumours: treatment outcomes at UKM medical centre. Malaysia. Asian Pac J Cancer Prev. 2012;13(6):2467–2471.

[16] Gouma E, Simos Y, Verginadis I, et al. A simple procedure for estimation of total body surface area and determination of a new value of Meeh's constant in rats. Lab Anim. 2012;46(1):40–45.
[17] Myers M, Britt KL, Wreford NG, et al. Methods for quantifying follicular numbers within the mouse ovary. Reproduction. 2004;127(5): 569–580.
[18] Ertas IE, Taskin S, Goklu R, et al. Long-term oncological and reproductive outcomes of fertility-sparing cytoreductive surgery in females aged 25 years and younger with malignant ovarian germ cell tumors. J Obstet Gynaecol Res. 2014;40(3):797–805.
[19] Satoh T, Aoki Y, Kasamatsu T, et al. Administration of standard-dose BEP regimen (bleomycin + etoposide + cisplatin) is essential for treatment of ovarian yolk sac tumour. Eur J Cancer. 2015;51(3):340–351.
[20] Kang H, Kim TJ, Kim WY, et al. Outcome and reproductive function after cumulative high-dose combination chemotherapy with bleomycin, etoposide and cisplatin (BEP) for patients with ovarian endodermal sinus tumor. Gynecol Oncol. 2008;111(1):106–110.
[21] Behringer K, Wildt L, Mueller H, et al. German Hodgkin Study Group. No protection of the ovarian follicle pool with the use of GnRH-analogues or oral contraceptives in young women treated with escalated BEACOPP for advanced-stage Hodgkin lymphoma. Final results of a phase II trial from the German Hodgkin Study Group. Ann Oncol. 2010;21(10):2052–2060.
[22] Reh A, Oktem O, Oktay K. Impact of breast cancer chemotherapy on ovarian reserve: a prospective observational analysis by menstrual history and ovarian reserve markers. Fertil Steril. 2008;90(5):1635–1639.
[23] McLaughlin M, Kelsey TW, Wallace WH, et al. Non-growing follicle density is increased following adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) chemotherapy in the adult human ovary. Hum Reprod. 2017; 32(1):165–174.
[24] Blumenfeld Z, Dann E, Avivi I, et al. Fertility after treatment for Hodgkin's disease. Ann Oncol. 2002;13 (Suppl. 1):138–147.
[25] Chapman RM, Sutcliffe SB. Protection of ovarian function by oral contraceptives in women receiving chemotherapy for Hodgkin's disease. Blood. 1981;58(4):849–851.
[26] Lambertini M, Moore HCF, Leonard RCF, et al. Gonadotropin-releasing hormone agonists during chemotherapy for preservation of ovarian function and fertility in premenopausal patients with early breast cancer: a systematic review and meta-analysis of individual patient-level data. J Clin Oncol. 2018;36(19):1981–1990.
[27] Gilani MM, Hasanzadeh M, Ghaemmaghami F, et al. Ovarian preservation with gonadotropin-releasing hormone analog during chemotherapy. Asia-Pacific J Clin Oncol. 2007;3(2):79–83.
[28] Demeestere I, Brice P, Peccatori FA, et al. No evidence for the benefit of gonadotropin-releasing hormone agonist in preserving ovarian function and fertility in lymphoma survivors treated with chemotherapy: final long-term report of a prospective randomized trial. J Clin Oncol. 2016;34(22):2568–2574.
[29] Elgindy EA, El-Haieg DO, Khorshid OM, et al. Gonadatrophin suppression to prevent chemotherapy-induced ovarian damage: a randomized controlled trial. Obstet Gynecol. 2013;121(1):78–86.
[30] Huang YH, Zhao XJ, Zhang QH, et al. The GnRH antagonist reduces chemotherapy-induced ovarian damage in rats by suppressing the apoptosis. Gynecol Oncol. 2009;112(2):409–414.
[31] Lemos CN, Reis FM, Pena GN, et al. Assessment of fertility protection and ovarian reserve with GnRH antagonist in rats undergoing chemotherapy with cyclophosphamide. Reprod Biol Endocrinol. 2010; 8(1):51.
[32] Meirow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update. 2001;7(6):535–543.
[33] Shahrokh Tehraninezhad E, Mehrabi F, Taati R, et al. Analysis of ovarian reserve markers (AMH, FSH, AFC) in different age strata in IVF/ICSI patients. IJRM. 2016;14(8):501–506.
[34] Anderson RA, Themmen APN, Qahtani AA, et al. The effects of chemotherapy and long-term gonadotrophin suppression on the ovarian reserve in premenopausal women with breast cancer. Hum Reprod. 2006;21(10):2583–2592.
[35] Steiner AZ, Pritchard D, Stanczyk FZ, et al. Association between biomarkers of ovarian reserve and infertility among older women of reproductive age. JAMA. 2017;318(14):1367–1376. Oct 10
[36] Fanchin R, Schonäuer LM, Righini C, et al. Serum anti-Müllerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. Hum Reprod. 2003;18(2):323–327.
[37] Barbakadze L, Kristesashvili J, Khonelidze N, et al. The correlations of anti-mullerian hormone, follicle-stimulating hormone and antral follicle count in different age groups of infertile women. Int J Fertil Steril. 2015;8(4):393–398.