Oncology Research, Vol. 22, pp. 283–292
Printed in the USA.

0965-0407/15 $90.00 + .00
DOI: http://dx.doi.org/10.3727/096504015X14410238486720
E-ISSN 1555-3906
www.cognizantcommunication.com

Mechanistic Study on Triptorelin Action in Protecting From 5-FU-Induced Ovarian Damage in Rats

Ying Wang,* Xiaoyu Tian,* Lingxia Liang,† Yan Wang,‡ Ruifang Wang,* Xiaolin Cheng,* Zhen Yan,* Yawei Chen,* and Pengwei Qi*

*Obstertrics and Gynaecology, The First Affiliated Hospital of Henan University of Science and Technology, Luoyang, China
†Medical Oncology, The First People's Hospital of Nanyang, Nanyang, China
‡Agricultural College, GuangXi University, Nanning, China

Triptorelin, a kind of GnRH agonist, is widely used in the treatment of hormone-responsive cancers in the clinic. This study aimed to discover the underlying mechanism of triptorelin in protection from 5-fluorouracil (5-FU)-induced ovarian damage in Sprague–Dawley rats. In the present study, after using 5-FU to induce ovarian damage in rats, body weight and wet ovaries were weighed, the levels of estradiol (E2), follicle-stimulating hormone (FSH), and anti-Müllerian hormone (AMH) in blood were detected, and the expression of Bcl-2, Bax, and NF-κB was determined. It suggested that, compared to the control, body weight gain, the ratio of ovarian wet weight to body weight, primary follicle numbers, and the levels of AMH were significantly decreased, while the concentration of E2 and FSH was heavily increased following 5-FU administration. In contrast, after coadministration of triptorelin with 5-FU, the ratio of ovarian wet weight to body weight and the levels of AMH were significantly increased, whereas the level of E2 and FSH was decreased significantly when compared with the 5-FU group. Furthermore, at indicated times, 5-FU led to the reduced Bcl-2 and NF-κB expression and increased Bax expression while triptorelin plus 5-FU increased Bcl-2 and NF-κB expression and decreased Bax expression. It was indicated that triptorelin could protect rats from 5-FU-induced ovarian damage by modulation of hormones, Bcl-2, Bax, and NF-κB. These results might highlight the mechanism of triptorelin as a protective agent in clinical chemotherapy for ovarian damage.

Key words: Triptorelin; 5-Fluorouracil (5-FU); Rat; Ovarian damage; Bcl-2; Bax; NF-κB

INTRODUCTION

Ovarian cancer is the most fatal disease affecting the female reproductive system (1). Unless diagnosed early, the prognosis for ovarian cancer is extremely poor because early ovarian cancer shows nonspecific symptoms (2). To date, surgery is the most preferred treatment choice for ovarian cancer, and chemotherapy is usually administered after surgery to treat any residual disease. To effectively control ovarian cancer, improve patient survival, and reduce disease incidence, novel approaches for the early detection, effective treatment, and prevention are urgently needed.

GnRH agonists are widely used for stimulating the recovery of ovarian damages. Previous studies proved that GnRH agonists had better safety profiles than estrogens and anti-androgens. In addition, slow-release formulations of GnRH agonists offer flexibility to patients, improve quality of life, and eventually reduce the cost of treatment (3). GnRH agonist has been welcomed both by patients and physicians because of the convenience of single administration. Triptorelin, a synthetic analog of GnRH agonists, is widely used in women undergoing controlled ovarian stimulation (COS) (4), including prevention of a premature LH surge and luteinization (5), eliciting a lower cancellation rate and improvement of follicular recruitment (6), allowing recovery of a larger number of oocytes (7) and conferring ovarian radioprotection to adult female rats (8). In a previous study, it was shown that 3.75 mg triptorelin could be used to preserve ovarian function in women treated with chemotherapy for early stage breast cancer (9,10). A half dose (1.87 mg) of depot triptorelin could be successfully used in ovarian stimulation for IVF and produced a higher number of good quality embryos with a good chance of implantation (11). Another study suggested that both full-dose (3.75 mg) and half-dose (1.87 mg) GnRH agonist triptorelin seemed to be equally effective in pituitary desensitization, with similar duration times for both desensitization and recovery (12). In a recent

Address correspondence to Xiaoyu Tian, Obstetrics and Gynaecology, The First Affiliated Hospital of Henan University of Science and Technology, No. 24 of Jinghua Road, Jianxi District, Luoyang, Henan Province, 471003 China. Tel/Fax: +8637969823006; E-mail: tianxiaoyu1415@163.com

review, Leone Roberti Maggiore et al. proved that triptorelin is one of the most commonly used GnRH agonists for the treatment of endometriosis (13). The in vitro results also indicated that triptorelin dose-dependently inhibited the proliferation of human breast and ovarian cancer cell lines (14). Furthermore, triptorelin inhibited estradiol-induced serum response element (SRE) activation, c-fos expression, and the proliferation in human endometrial, ovarian, and breast cancer cells (15). However, whether triptorelin exerts a protective function in 5-fluorouracil (5-FU)-induced ovarian damage in Sprague–Dawley rats remains unclear. Therefore, the present study specifically addresses concerns about the protective function of triptorelin in ovarian reserve. The research results will benefit female cancer patients treated with triptorelin and will be helpful for the clinical application of chemotherapy protective agents.

MATERIALS AND METHODS

Animals and Treatments

Sixty adult female Sprague–Dawley (SD) rats aged 50 days were purchased from Tongji Medical College of Huazhong University of Science and Technology Experimental Animal Center (Permit No: SCXK-2010-0007). All procedures were performed in accordance with the Health Guide for the Care and Use of Laboratory Animals of China. Rats were randomly divided into four groups (10 rats per group): normal saline control (NSC), triptorelin (T, 0.1 mg/kg), 5-FU (5-FU, 80.0 mg/kg), 5-FU plus triptorelin (5-FU+T, 80.0 mg/kg + 0.1 g/kg). 5-FU and triptorelin, purchased from Ipsen Pharma Biotech, France, were diluted in normal saline immediately before use. The rats in four groups received daily intraperitoneal injection of normal saline, 5-FU, triptorelin, or 5-FU plus triptorelin, respectively, for 1 week.

Histological Evaluation of Ovarian Tissue

At the end of the animal experiments, the rats were killed by an overdose of 10% chloral hydrate (5 ml/kg), and left and right ovaries were randomly weighed and assigned to be fixed in 4% paraformaldehyde (pH 7.3) and then embedded in paraffin. Paraffin-embedded ovarian tissues were serially sectioned at 3 μm for hematoxylin and eosin (H&E) staining and immunohistochemistry. The number of primordial and primary follicles were counted and compared according to a previous study (16).

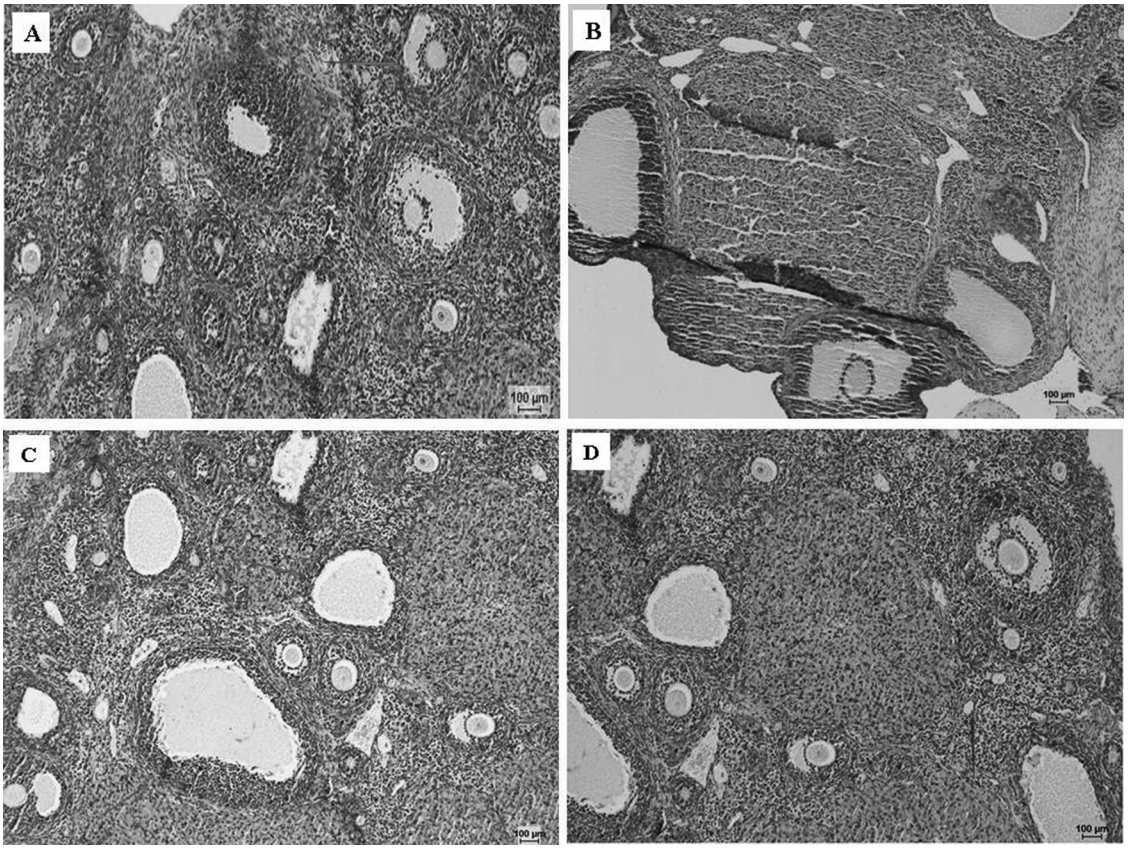

Figure 1. Effects of triptorelin on 5-FU-induced ovarian damage in rats. After being treated with normal saline (A), triptorelin (B), 5-FU (C), and 5-FU plus triptorelin (D), the structure of the ovary was determined.

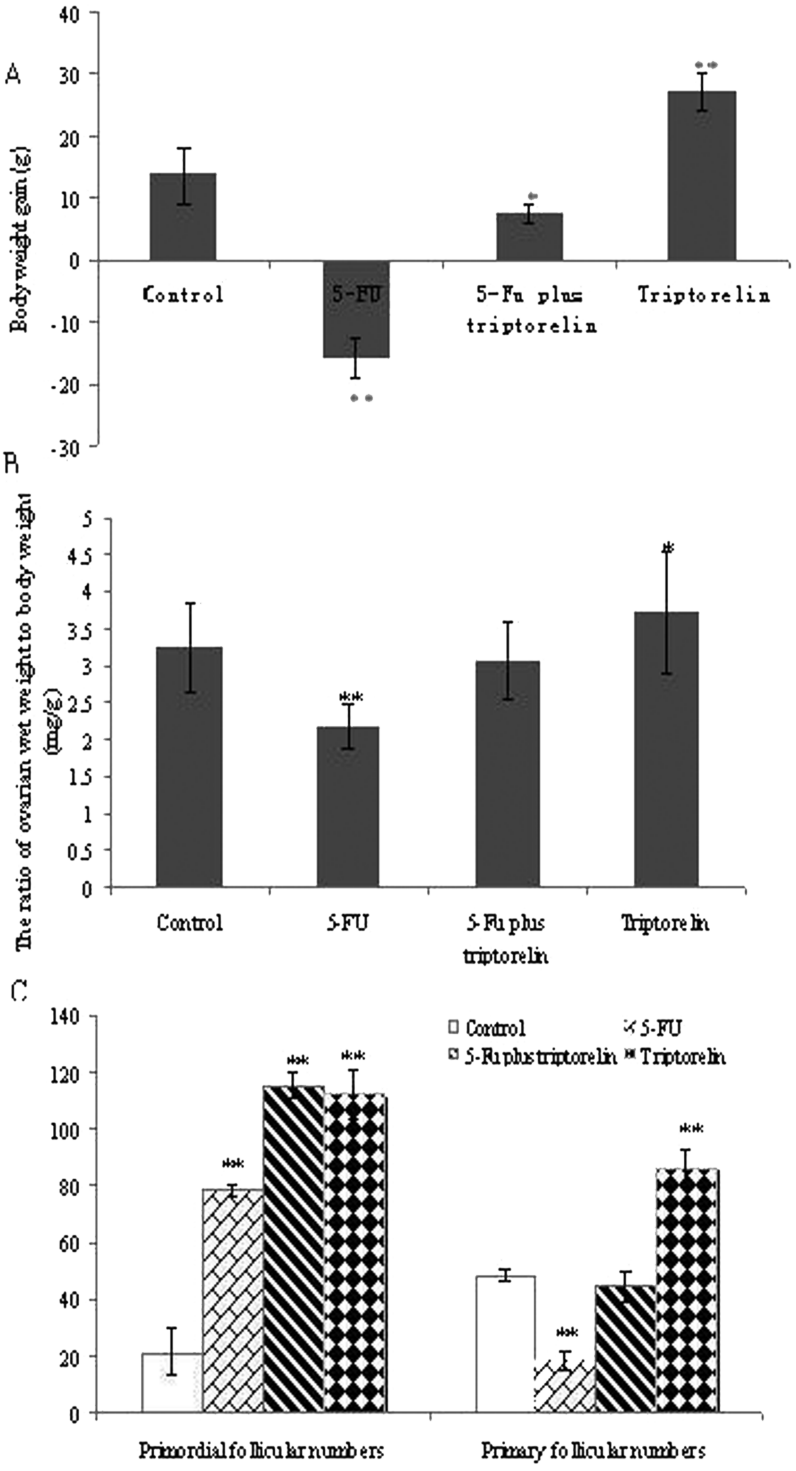

Figure 2. Effects of triptorelin on 5-FU-induced changes in body weight gain, the ratio of ovarian wet weight to body weight, and follicle numbers. After being treated with normal saline, 5-FU, 5-FU plus triptorelin, and triptorelin, body weight gain (A), the ratio of ovarian wet weight to body weight (B), and follicle numbers (C) were detected. *$p<0.05$, **$p<0.01$, or $p>0.05$ versus control.

Immunohistochemistry

Immunohistochemical staining was performed to detect the expression of Bax and Bcl-2 in paraffin-embedded tissue sections of rat ovaries using a standard protocol of horseradish peroxidase (HRP)-conjugated rabbit anti-human IgG described previously (17). The whole tissue slides from the selected cohort were stained with an automated procedure. The primary antibodies anti-Bax and anti-Bcl-2 (Cell Signaling Technology, Danvers, MA, USA) were manually applied at 1:1,000 and 1:2,000 dilutions, and the slides were incubated at 37°C for 1 h. Amplification and detection were performed using the UltraView Amplification

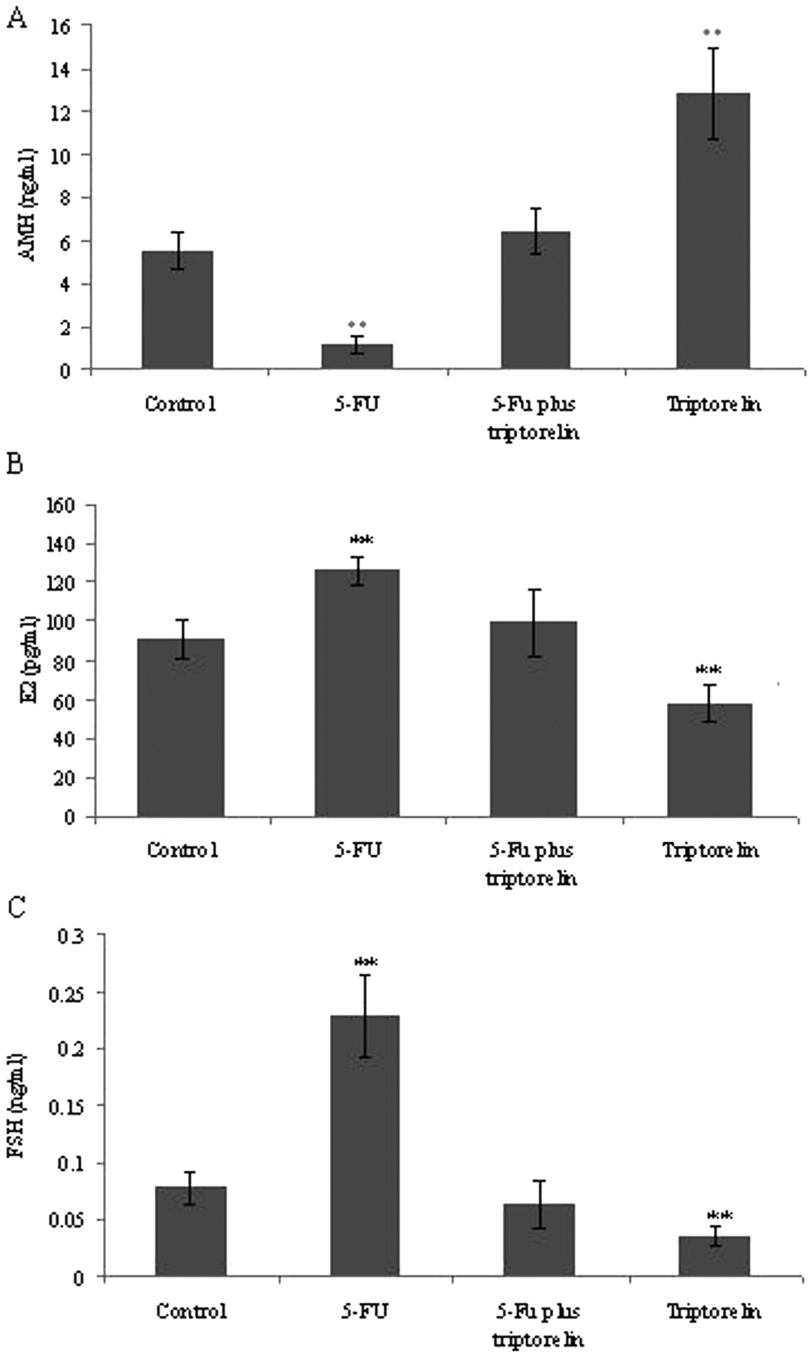

Figure 3. Effects of triptorelin on 5-FU-induced changes in hormone concentration in the blood of rats. After being treated with normal saline, 5-FU, 5-FU plus triptorelin, and triptorelin, the concentration of AMH (A), E2 (B), and FSH (C) in the blood of rats was measured. *$p<0.05$, **$p<0.01$, or $p>0.05$ versus control.

and DAB detection kits. The slides were counterstained with hematoxylin for 4 min and post-counterstained with bluing agent for 4 min. The slides were then washed with mild detergent and dehydrated in a series of 70% to 100% alcohol baths, cleared in a xylene bath, and cover slipped to analyze the average integrated optical density (IOD) of Bax and Bcl-2 staining using Image-pro plus 6.0 software (Media Cybernetics, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was performed to detect FSH, E2, and AMH levels in blood samples of rats with or without drug treatments. Briefly, the blood samples were obtained from the rats, isolated, and then centrifuged for ELISA analyses of FSH, E2, and AMH expression using a kit from Uscn Life Science Inc. (Wuhan, China) according to the manufacturer's protocol. Optical densities were read at 405 nm, and AMH concentrations were determined by comparison with standard curves.

Western Blot Analysis

Bcl-2/Bax and NF-κB expression was analyzed by the Western blot method. Briefly, proteins were collected and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred electrophoretically to PVDF membranes. The membranes were incubated at 4°C overnight with polyclonal anti-Bcl-2/Bax and anti-NF-κB (Cell Signaling Technology, Beverly, MA, USA) and immunoblotted with HRP-conjugated anti-rabbit IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 37°C for 45 min. The membranes were then developed with enhanced chemiluminescence (ECL) substrate (Beyotime, China) and exposed to X-ray film. Band density was quantitated using ImageJ software (ImageJ 1.35; National Institute Health, Bethesda, MD, USA). β-Actin was used to ensure adequate sample loading for all Western blots.

Real-Time RT-PCR

Expression of NF-κB in ovarian tissues was determined by RNA preparation and quantitative reverse transcription-polymerase chain reaction (RT-PCR). Briefly, total cellular RNA was isolated from cells on six-well plates using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The real-time RT-PCR analysis was carried out using the QuantiTect SYBR Green RT-PCR Kit (Qiagen, Valencia, CA, USA) under the ABI Prism 7500 Sequence Detector (Applied Biosystems, Foster City, CA, USA). The reaction run at one cycle of 50°C for 2 min and 94°C for 15 min, followed by 40 cycles of 94°C for 15 s, 55°C for 30 s and 72°C for 30 s. We used β-actin expression as an internal control. Specific primer sequences were synthesized in BIOSUNE Biological Technology Corp (Shanghai, China), and the sequences of the primers were as follows: β-actin, sense: 5′-GACATGCGCCTCGGAGAAAC-3′, antisense: 5′-AGCCGCAGATGCGCTTAAGT-3′; NF-κB, sense: 5′-TCGTTGGCTTTGTCTCTCC-3′, antisense: 5′-CCACCTCCGGGACGCTGTCTAC-3′.

Statistical Analysis

Statistical analysis was carried out with one-way ANOVA and assessed for statistical difference by one-way analysis of variance (ANOVA) using SPSS17.0 software. Values are expressed as means ± SD. The mean values and standard deviations were calculated from three independent experiments. Differences were considered statistically significant at $p<0.05$ and $p<0.01$.

RESULTS

Protective Effects of Triptorelin on 5-FU-Induced Ovarian Damage of Rats

Results of the protective effects of triptorelin on 5-FU-induced ovarian damage of rats were evaluated. As shown in Figure 1, after 5-FU administration (Fig. 1B), the structure of the ovary was damaged in comparison with the NSC group (Fig. 1A) and the T group (Fig. 1D), and triptorelin prevented 5-FU-induced ovarian structure damage (Fig. 1C). Additionally, 5-FU resulted in the loss of body weight (Fig. 2A), the ratio of ovarian wet weight to body weight (Fig. 2B), and primary follicle numbers (Fig. 2C) significantly ($p<0.05$), while triptorelin could eliminate the harmful effects induced by 5-FU.

Effect of Triptorelin on 5-FU-Induced Changes of Hormone Secretion in Blood of Rats

Results in Figure 3 showed that, compared with the NCS group, the AMH level was decreased significantly and increased in the 5-FU group, while it was promoted in the T group ($p<0.01$) (Fig. 3A). However, E2 (Fig. 3B) and FSH (Fig. 3C) levels in the 5-FU group were significantly higher, and they were lower in the T group when compared with the NCS group, respectively ($p<0.01$). Furthermore, E2, AMH, and FSH levels between the 5-FU plus triptorelin group and the NCS group showed no difference ($p>0.05$), indicating that triptorelin could protect ovaries from damage by changing the secretion of hormones.

Effect of Triptorelin on 5-FU-Induced Changes of Bax and Bcl-2

The influence of triptorelin on 5-FU-induced changes of apoptosis-related genes Bax and Bcl-2 was detected by immunohistochemistry and Western blot, and the results are shown in Figures 4 and 5. It demonstrated that the IOD value of Bax was significantly promoted in the 5-FU group (Fig. 4B) and obviously inhibited in the T group (Fig. 4D) in comparison with the NCS group (Fig. 4A)

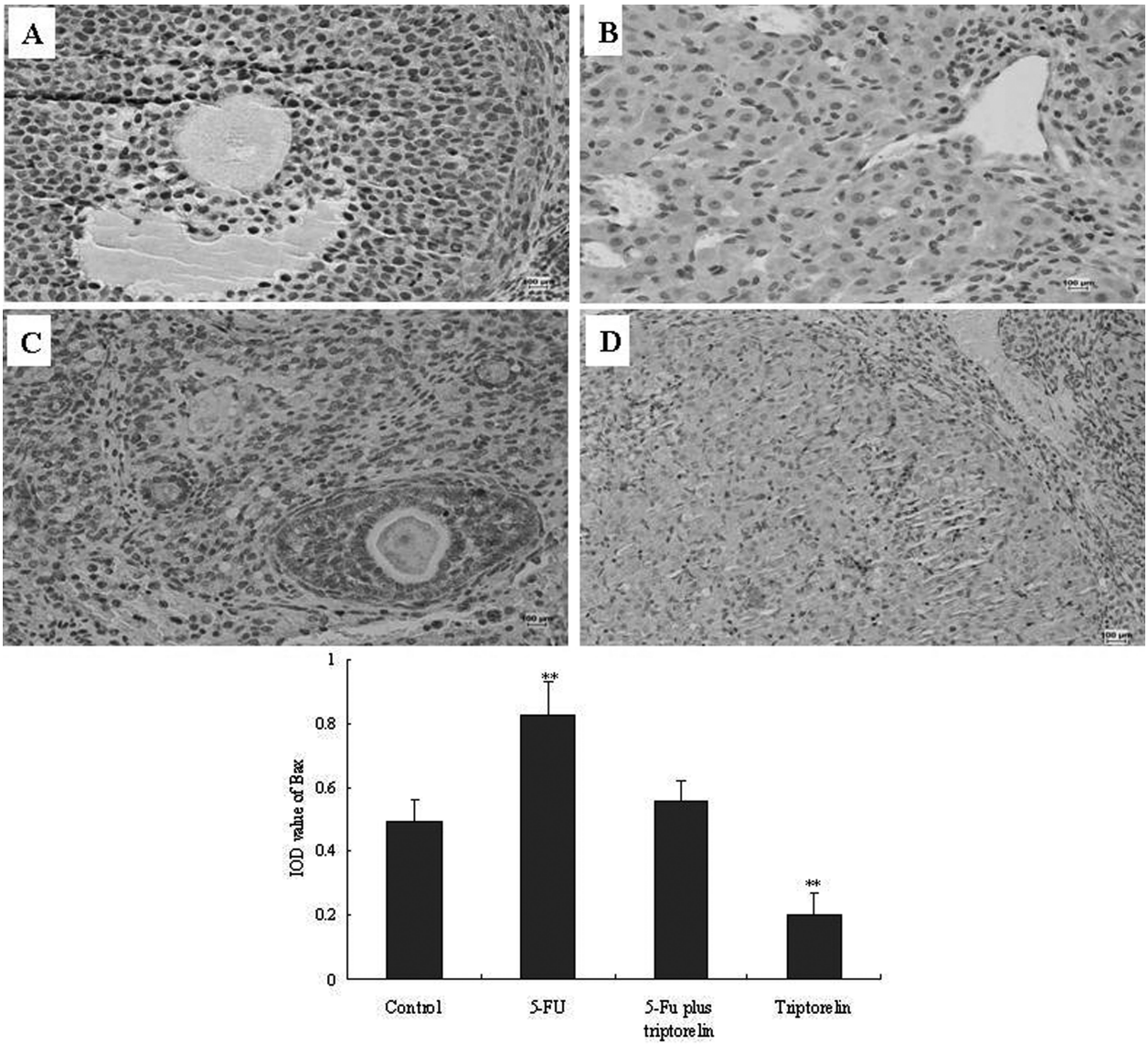

Figure 4. Effect of triptorelin on 5-FU-induced changes of Bax. After being treated with normal saline (A), 5-FU (B), 5-FU plus triptorelin (C), and triptorelin (D), the percentage of Bax-positive cells was determined by immunohistochemical staining. *$p<0.05$, **$p<0.01$, or $p>0.05$ versus control.

($p<0.05$), and the increase could be decreased by triptorelin (Fig. 4C). Accordingly, IOD values of Bcl-2 in the four groups shown in Figure 5 suggested that, compared to the control (Fig. 5A), 5-FU downregulated (Fig. 5B), while triptorelin upregulated, the IOD value of Bcl-2 (Fig. 5D). Triptorelin further increased 5-FU-induced downregulation of Bcl-2 (Fig. 5C) ($p<0.05$). Furthermore, protein expression of Bax was upregulated, and Bcl-2 was downregulated, in the 5-FU group, and the effects could be inhibited by triptorelin (Fig. 5E). The findings above suggest that triptorelin can inhibit cell apoptosis induced by 5-FU.

Effect of Triptorelin on 5-FU-Induced Changes of the NF-κB Signaling

The quantitative analysis for NF-κB in four groups is presented in Figure 6. This indicated that there was no significant difference in mRNA expression of NF-κB analyzed by RT-PCR between four groups ($p>0.05$) (Fig. 6A). Consistent with the findings above, protein expression of NF-κB also showed no changes in the four groups. Moreover, the expression of phospho-NF-κB was detected. It was suggested that the expression of phospho-NF-κB in 5-FU was significantly less than that in the NSC group ($p<0.05$), and a significant increase in phospho-NF-κB expression was

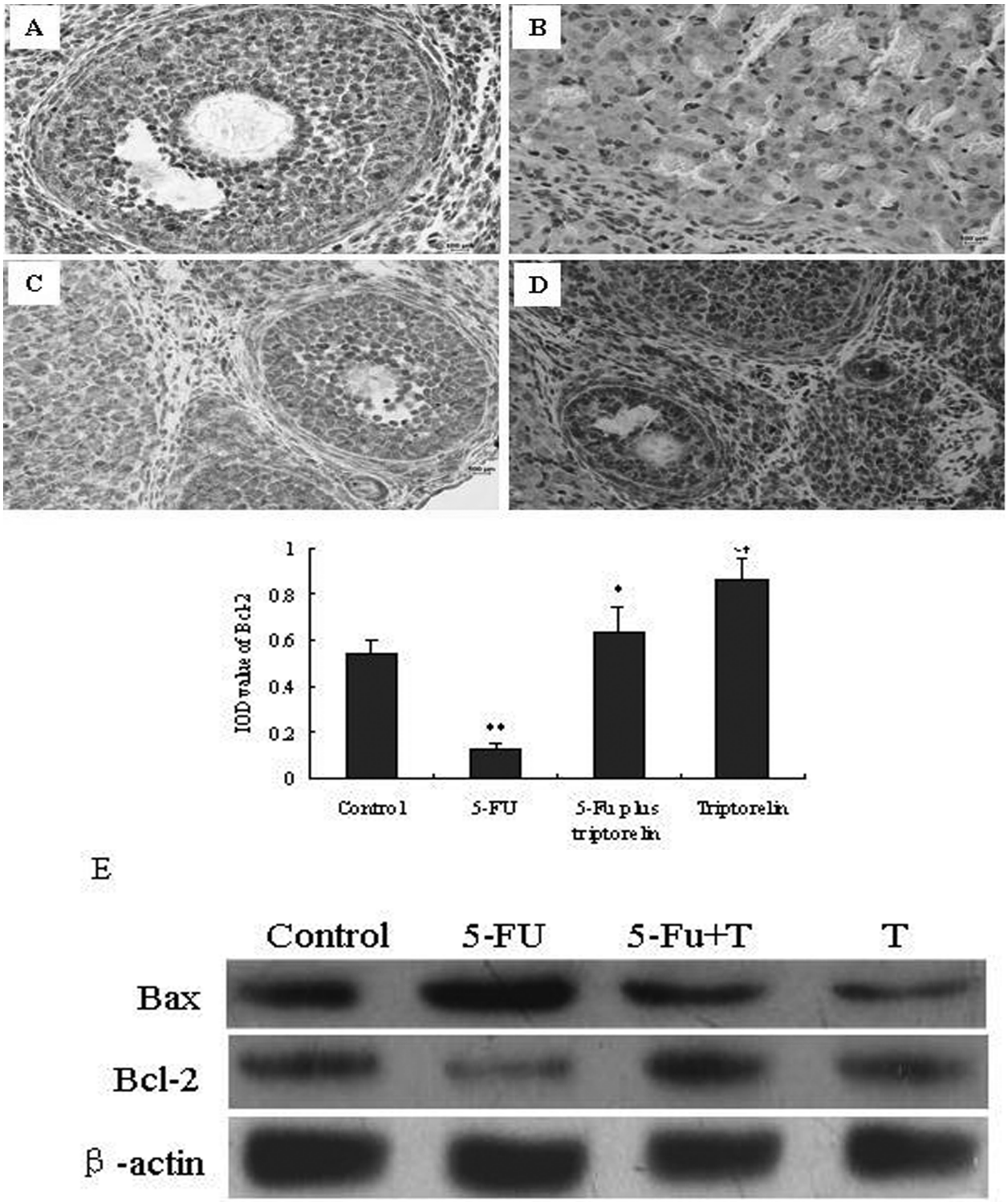

Figure 5. Effect of triptorelin on 5-FU-induced changes of Bcl-2. After being treated with normal saline (A), 5-FU (B), 5-FU plus triptorelin (C), and triptorelin (D), the percentage of Bcl-2-positive cells was determined by immunohistochemical staining. Protein expression of Bax and Bcl-2 was measured by Western blot (E). *$p<0.05$, **$p<0.01$, or $p>0.05$ versus control.

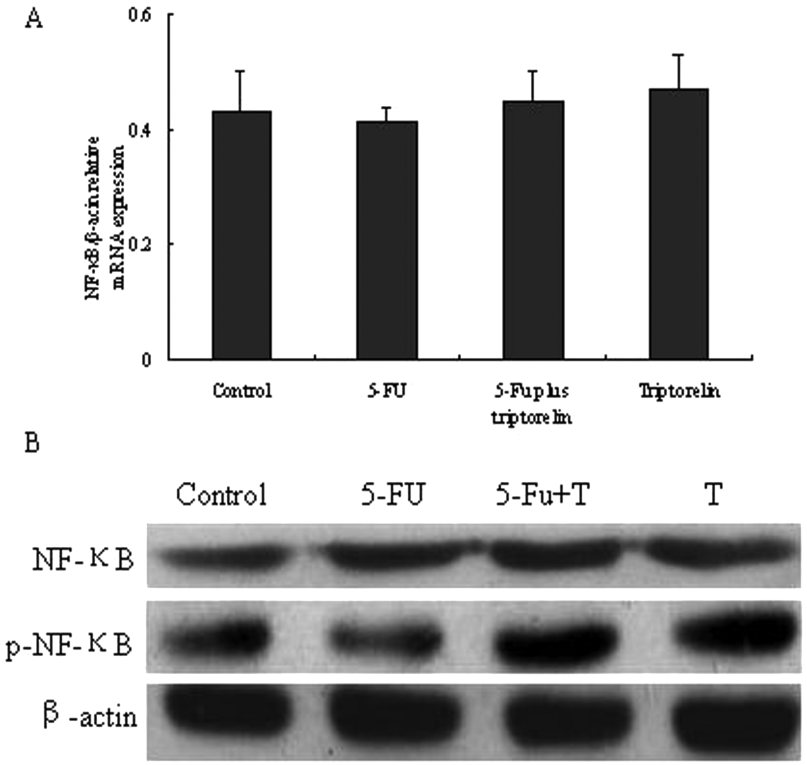

Figure 6. Effect of triptorelin on 5-FU-induced changes of NF-κB. After being treated with normal saline, 5-FU, 5-FU plus triptorelin, or triptorelin, mRNA expression of NF-κB was analyzed using RT-PCR (A). NF-κB and phospho-NF-κB protein expression was determined by Western blot (B). *$p<0.05$, **$p<0.01$, or $p>0.05$ versus control.

observed in the T group ($p<0.05$) (Fig. 6B). This indicated that phospho-NF-κB expression was decreased in response to 5-FU, but the reduction of phospho-NF-κB was restored by triptorelin.

DISCUSSION

GnRH agonists such as triptorelin are well known for their benefits in controlling ovarian stimulation (COS) (7). 5-FU has been reported as a novel approach for the treatment of various types of cancer, including colon cancer, rectal cancer, breast cancer, and esophageal cancer (18–21). Triptorelin in combination with cisplatin increased anti-ovarian cancer activity and survival of Wistar rats (22). However, the correlation between the GnRH agonist triptorelin and 5-FU sensitivity remains controversial. In the present study, it was found that triptorelin could protect rats from 5-FU-induced ovarian damage by changing the secretion of hormones and the expression of Bcl-2, Bax, and NF-κB.

A previous study suggested that both FSH and luteinizing hormone (LH) were required for normal follicular growth and maturation (23). Triptorelin could successfully be used to reduce pituitary suppression and lower the cost in a long protocol for in vitro fertilization (24). Janssens et al. first reported that a dose of short-acting daily triptorelin resulted in a greater suppression of endogenous LH and FSH during the follicular phase (25). Several studies have also shown that a half-dose triptorelin injection is enough for the prevention of LH surges in patients stimulated with human menopausal gonadotropin (hMG) (11,26). Treatment with triptorelin could reduce estradiol concentrations in cultured human granulosa luteinized cells (27) and in premenopausal women (28). A significant decrease in FSH, LH, and E2 levels between patient groups was observed after the 7th or 12th week of triptorelin treatment (29,30). However, a recently randomized trial showed that administration of triptorelin on postnatal days 12–14 increased the levels of LH mRNA in the pituitary gland and the weight of testes (31). This study also demonstrated that 5-FU administration led to the decrease in body weight, ovarian wet weight, and follicle numbers and the increase in the levels of E2 and FSH. In contrast, after coadministration of triptorelin with 5-FU, body weight, ovarian wet weight, and follicle numbers were significantly increased, whereas the levels of E2 and FSH were decreased significantly.

AMH, a member of the TGF-β superfamily, is produced by granulose cells of small-growing nonatretic follicles in rat and mouse ovaries (32). Usually, AMH is considered to be a reliable marker for ovarian reserve assessment and is often used as an indicator of ovarian damage (33). A study

reported that AMH was negatively correlated with FSH in different age groups of infertile women (34). Other investigators have suggested that AMH inhibits recruitment of primordial follicles into the growing pool, while at cyclic recruitment, AMH lowers the FSH sensitivity of follicles (35,36). Results obtained from AMH knockout mice demonstrated that AMH regulated the development of early follicles in two ways, as a negative stimulator of follicular maturation and as an inhibitor of FSH sensitivity of growing follicles, serving to negatively modulate the FSH-dependent selection of the dominant follicles (37). Another study showed that pituitary desensitization with GnRH agonists resulted in a significant increase in AMH levels, which might explain the enhanced ovarian response to conventional COS (4). Results here showed that the expression of AMH was significantly decreased when 5-FU was administered, indicating damage of ovarian follicles induced by 5-FU. Moreover, AMH was increased after being treated with triptorelin in combination with 5-FU, showing the protection of triptorelin in 5-FU-induced ovary damage.

Bax and Bcl-2, the major members of Bcl-2 family, play a key role in tumor progression or inhibition of intrinsic apoptotic pathway triggered by mitochondrial dysfunction (38). Although it has been suggested that activation of NF-κB is not directly associated with tumor development and progression (39), NF-κB has been considered to be a major biomarker and therapeutic target (40). An investigation has suggested that 5-FU resistance may also be induced by p53 gene mutation and overexpression of antiapoptotic factors (41). The present study indicated that Bax and NF-κB were upregulated, and Bcl-2 was downregulated, by 5-FU, and the effects could be inhibited by triptorelin. The findings above proved that triptorelin could inhibit cell apoptosis induced by 5-FU.

In conclusion, this study provided evidence that a high dose of 5-FU led to ovarian damage by increasing E2, FSH, Bax, and NF-κB levels and decreasing follicle numbers and the levels of AMH and Bcl-2. Triptorelin can protect 5-FU-induced ovarian damage through promoting follicle numbers and AMH, Bcl-2, and phospho-NF-κB levels, inhibiting E2, FSH, and Bax levels.

ACKNOWLEDGEMENTS: This work was supported by the Medical Science and Technology Project of Henan Province (1201052A-3). We also give our thanks to Jiangang Wang, Shoumin Xi, Huili Liu, Pengfei Zhang, and Xiangjun Qiu in Henan University of Science and Technology for their generous help. The authors declare no conflicts of interest.

REFERENCES

1. Jemal, A.; Siegel, R.; Ward, E.; Hao, Y.; Xu, J.; Murray, T.; Thun, M. J. Cancer statistics, 2008. CA Cancer J. Clin. 58:71–96; 2008.
2. Didžiapetrienė, J.; Bublevič, J.; Smailytė, G.; Kazbarienė, B.; Stukas, R. Significance of blood serum catalase activity and malondialdehyde level for survival prognosis of ovarian cancer patients. Medicina (Kaunas). 50:204–208; 2014.
3. Ploussard, G.; Mongiat-Artus, P. Triptorelin in the management of prostate cancer. Future Oncol. 9:93–102; 2013.
4. Jayaprakasan, K.; Campbell, B. K.; Hopkisson, J. F.; Clewes, J. S.; Johnson, I. R.; Raine-Fenning, N. J. Effect of pituitary desensitization on the early growing follicular cohort estimated using anti-Mullerian hormone. Hum. Reprod. 23:2577–2583; 2008.
5. Karatekeli, E.; Ozörnek, H.; Jamal, H.; Ergin, E.; Ongun, B. Administration of alternate day triptorelin for controlled ovarian hyperstimulation is cost effective. Fertil. Steril. 85:518–519; 2006.
6. Itskovitz-Eldor, J.; Kol, S.; Mannaerts, B. Use of a single bolus of GnRH agonist triptorelin to trigger ovulation after GnRH antagonist ganirelix treatment in women undergoing ovarian stimulation for assisted reproduction, with special reference to the prevention of ovarian hyperstimulation syndrome: preliminary report: Short communication. Hum. Reprod. 15: 1965–1968; 2000.
7. Liu, H. C.; Lai, Y. M.; Davis, O.; Graf, M.; Grifo, J.; Cohen, J.; Rosenwaks, Z. Improved pregnancy outcome with gonadotrophin releasing hormone agonist stimulation is due to the improvement in oocytes quantity rather than quality. J. Assist. Reprod. Genet. 9:338–342; 1992.
8. Camats, N.; García, F.; Parrilla, J. J.; Calaf, J.; Martín-Mateo, M.; Caldés, M. G. The GnRH analogue triptorelin confers ovarian radio-protection to adult female rats. Mutat. Res. 669:67–79; 2009.
9. Del Mastro, L.; Boni, L.; Michelotti, A.; Gamucci, T.; Olmeo, N.; Gori, S.; Giordano, M.; Garrone, O,.; Pronzato, P.; Bighin, C.; Levaggi, A.; Giraudi, S.; Cresti, N.;, Magnolfi, E.; Scotto, T.; Vecchio, C.; Venturini, M. Effect of the gonadotropin-releasing hormone analogue triptorelin on the occurrence of chemotherapy-induced early menopause in premenopausal women with breast cancer: A randomized trial. JAMA 306:269–276; 2011.
10. Munster, P. N.; Moore, A. P.; Ismail-Khan, R.; Cox, C. E.; Lacevic, M.; Gross-King, M.; Xu, P.; Carter, W. B.; Minton, S. E. Randomized trial using gonadotropin-releasing hormone agonist triptorelin for the preservation of ovarian function during (neo)adjuvant chemotherapy for breast cancer. J. Clin. Oncol. 30:533–538; 2012.
11. Dal Prato, L.; Borini, A.; Coticchio, G.; Cattoli, M.; Flamigni, C. Half-dose depot triptorelin in pituitary suppression for multiple ovarian stimulation in assisted reproduction technology: A randomized study. Hum. Reprod. 19:2200–2205; 2004.
12. Balasch, J.; Gómez, F.; Casamitjana, R.; Carmona, F.; Rivera, F.; Vanrell, J. A. Pituitary-ovarian suppression by the standard and half-doses of D-Trp-6-luteinizing hormone-releasing hormone depot. Hum. Reprod. 7:1230–1234; 1992.
13. Leone Roberti Maggiore, U.; Scala, C.; Remorgida, V.; Venturini, P. L.; Del Deo, F.; Torella, M.; Colacurci, N.; Salvatore, S.; Ferrari, S.; Papaleo, E.; Candiani, M.; Ferrero, S. Triptorelin for the treatment of endometriosis. Expert Opin. Pharmacother. 15:1153–1179; 2014.
14. Saleh-Abady, M. M.; Alizadeh, A.; Shamsipour, F.; Naderi-Manesh, H. The anticancer activity compared between triptorelin and a new gonadotropin releasing hormone analogue. Avicenna. J. Med. Biotechnol. 1:105–110; 2009.
15. Gründker, C.; Günthert, A. R.; Hellriegel, M.; Emons, G. Gonadotropin-releasing hormone (GnRH) agonist triptorelin inhibits estradiol-induced serum response element

(SRE) activation and c-fos expression in human endometrial, ovarian and breast cancer cells. Eur. J. Endocrinol. 151:619–628; 2004.
16. Myers, M.; Britt, K. L.; Wreford, N. G.; Ebling, F. J.; Kerr, J. B. Methods for quantifying follicular numbers within the mouse ovary. Reproduction 127:569–580; 2004.
17. Gründker, C.; Föst, C.; Fister, S.; Nolte, N.; Günthert, A. R.; Emons, G. Gonadotropin-releasing hormone type II antagonist induces apoptosis in MCF-7 and triple-negative MDA-MB-231 human breast cancer cells in vitro and in vivo. Breast Cancer Res. 12:R49; 2010.
18. Iacopetta, B.; Kawakami, K.; Watanabe, T. Predicting clinical outcome of 5-fluorouracil-based chemotherapy for colon cancer patients: Is the CpG island methylator phenotype the 5-fluorouracil-responsive subgroup? Int. J. Clin. Oncol. 13:498–503; 2008.
19. Liu, J.; Wang, Z.; Wu, K.; Li, J.; Chen, W.; Shen, Y.; Guo, S. Paclitaxel or 5-fluorouracil/esophageal stent combinations as a novel approach for the treatment of esophageal cancer. Biomaterials 53:592–599; 2015.
20. Patel, P. A. Evolution of 5-fluorouracil-based chemoradiation in the management of rectal cancer. Anticancer Drugs 22:311–316; 2011.
21. Yuan, Z.; Qu, X.; Wang, Y.; Zhang, D. Y.; Luo, J. C.; Jia, N.; Zhang, Z. T. Enhanced antitumor efficacy of 5-fluorouracil loaded methoxy poly (ethylene glycol)-poly (lactide) nanoparticles for efficient therapy against breast cancer. Colloids Surf. B. Biointerfaces 128:489–497; 2015.
22. Tkalia, I. G.; Vorobyova, L. I.; Grabovoy, A. N.; Svintsitsky, V. S.; Tarasova, T. O. The antitumor efficacy of cisplatin in combination with triptorelin and exemestane therapy for an ovarian cancer ascites model in Wistar rats. Exp. Oncol. 37:30–35; 2015.
23. Altermatt, J. L.; Marolf, A. J.; Wrigley, R. H.; Carnevale, E. M. Effects of FSH and LH on ovarian and follicular blood flow, follicular growth and oocyte developmental competence in young and old mares. Anim. Reprod. Sci. 133:191–197; 2012.
24. Li, Y.; Yang, D.; Zhang, Q. Clinical outcome of one-third-dose depot triptorelin is the same as half-dose depot triptorelin in the long protocol of controlled ovarian stimulation. J. Hum. Reprod. Sci. 5:14–19; 2012.
25. Janssens, R. M.; Lambalk, C. B.; Vermeiden, J. P.; Schats, R.; Bernards, J. M.; Rekers-Mombarg, L. T.; Schoemaker, J. Dose-finding study of triptorelin acetate for prevention of a premature LH surge in IVF: A prospective, randomized, double-blind, placebo-controlled study. Hum. Reprod. 15:2333–2340; 2000.
26. Kulikowski, M.; Wołczyński, S.; Grochowski, D.; Kuczyński, W.; Sola, E.; Szamatowicz, J.; Syrewicz, M.; Domitrz, J.; Szamatowicz, M. "Long protocol" for ovarian stimulation using D-Trp6 LHRH (Triptorelin Depot) and menopausal gonadotropin in the IVF-ET program. Ginekol. Pol. 67:243–247; 1996.
27. Metallinou, C.; Köster, F.; Diedrich, K.; Nikolettos, N.; Asimakopoulos, B. The effects of cetrorelix and triptorelin on the viability and steroidogenesis of cultured human granulosa luteinized cells. In Vivo 26:835–839; 2012.
28. Jannuzzo, M. G.; Di Salle, E.; Spinelli, R.; Pirotta, N.; Buchan, P.; Bello, A. Estrogen suppression in premenopausal women following 8 weeks of treatment with exemestane and triptorelin versus triptorelin alone. Breast Cancer Res. Treat. 113:491–499; 2009.
29. Li, Z.; Zhang, H. Y.; Zhu, Y. J.; Hu, Y. J.; Qu, P. P. A randomized study comparing the side effects and hormonal status of triptorelin and leuprorelin following conservative laparoscopic surgery for ovarian endometriosis in Chinese women. Eur. J. Obstet. Gynecol. Reprod. Biol. 183:164–168; 2014.
30. Parsanezhad, M. E.; Azmoon, M.; Alborzi, S.; Rajaeefard, A.; Zarei, A.; Kazerooni, T.; Frank, V.; Schmidt, E. H. A randomized, controlled clinical trial comparing the effects of aromatase inhibitor (letrozole) and gonadotropin-releasing hormone agonist (triptorelin) on uterine leiomyoma volume and hormonal status. Fertil. Steril. 93:192–198; 2010.
31. Dygalo, N. N.; Shemenkova, T. V.; Kalinina, T. S.; Shishkina, G. T. Effects of gonadoliberin analogue triptorelin on the pituitary-testicular complex in neonatal rats. Bull. Exp. Biol. Med. 156:470–472; 2014.
32. Dresser, D. W.; Guerrier, D. Candidate Sertoli cell specific promoter element for a TGFbeta family member (Amh) and a 3' UTR enhancer/repressor for the same gene. Gene 363:159–165; 2005.
33. Peluso, C.; Fonseca, F. L.; Rodart, I. F.; Cavalcanti, V.; Gastaldo, G.; Christofolini, D. M.; Barbosa, C. P.; Bianco, B. AMH: An ovarian reserve biomarker in assisted reproduction. Clin. Chim. Acta 437:175–182; 2014.
34. Barbakadze, L.; Kristesashvili, J.; Khonelidze, N.; Tsagareishvili, G. The correlations of anti-mullerian hormone, follicle-stimulating hormone and antral follicle count in different age groups of infertile women. Int. J. Fertil. Steril. 8:393–398; 2015.
35. Gleicher, N.; Weghofer, A.; Barad, D. H. Discordances between follicle stimulating hormone (FSH) and anti-Müllerian hormone (AMH) in female infertility. Reprod. Biol. Endocrinol. 8:64; 2010.
36. Panidis, D.; Katsikis, I.; Karkanaki, A.; Piouka, A.; Armeni, A. K.; Georgopoulos, N. A. Serum anti-Müllerian hormone (AMH) levels are differentially modulated by both serum gonadotropins and not only by serum follicle stimulating hormone (FSH) levels. Med. Hypotheses 77:649–653; 2011.
37. Tsakos, E.; Tolikas, A.; Daniilidis, A.; Asimakopoulos, B. Predictive value of anti-müllerian hormone, follicle-stimulating hormone and antral follicle count on the outcome of ovarian stimulation in women following GnRH-antagonist protocol for IVF/ET. Arch. Gynecol. Obstet. 290:1249–1253; 2014.
38. Wang, Y.; Yin, R. F.; Teng, J. S. Wogonoside induces cell cycle arrest and mitochondrial mediated apoptosis by modulation of Bcl-2 and Bax in osteosarcoma cancer cells. Int. J. Clin. Exp. Pathol. 8:63–72; 2015.
39. DiDonato, J. A.; Mercurio, F.; Karin, M. NF-κB and the link between inflammation and cancer. Immunol. Rev. 246:379–400; 2012.
40. Uetsuka, H.; Haisa, M.; Kimura, M.; Gunduz, M.; Kaneda, Y.; Ohkawa, T.; Takaoka, M.; Murata, T.; Nobuhisa, T.; Yamatsuji, T.; Matsuoka, J.; Tanaka, N.; Naomoto, Y. Inhibition of inducible NF-κB activity reduces chemoresistance to 5-fluorouracil in human stomach cancer cell line. Exp. Cell. Res. 289:27–35; 2003.
41. Tominaga, T.; Iwahashi, M.; Takifuji, K.; Hotta, T.; Yokoyama, S.; Matsuda, K.; Higashiguchi, T.; Oku, Y.; Nasu, T.; Yamaue, H. Combination of p53 codon 72 polymorphism and inactive p53 mutation predicts chemosensitivity to 5-fluorouracil in colorectal cancer. Int. J. Cancer 126:1691–1701; 2010.