Apoptotic Effects of High Estradiol Concentrations on Endometrial Glandular Cells

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Context: High serum estradiol (E2) concentrations result in adverse reproductive outcome in in vitro fertilization cycles, and the detrimental effects are probably due to impaired endometrial receptivity.

Objective: Endometrial glandular cells (EGCs) are the cells that embryos first interact with during implantation. Our objective is to examine the in vitro EGC alterations after high E2 treatment.

Design: This was a prospective study.

Setting: The study was conducted at a tertiary university hospital.

Patients: Six women in the follicular phase participated in the study.

Interventions: EGCs were purified from human endometrium and cultured with different concentrations (0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-5}, 10^{-4} M) of E2.

Main Outcome Measure(s): EGC apoptosis and its underlying mechanism were measured.

Results: In vitro BeWo spheroid-EGC implantation assay demonstrated that the stimulation with 10^{-5} and 10^{-4} M E2 for 2 days decreased embryo implantation potentials. Presence of apoptotic bodies and DNA fragmentation and an increased percentage of sub-G1 phase were found in EGCs treated with high E2 concentrations. The high E2-treated EGCs could be rescued from apoptosis after the addition of estrogen receptor antagonist ICI 182 780. Western blot revealed increased inhibitory-κB (IκB)-α expression and decreased nuclear factor-κB (NF-κB) expression in high E2-treated EGCs, and NF-κB binding site-driven luciferase activity was decreased as well. When EGCs were pretreated with IκB-α small interfering RNA, high E2-induced B cell lymphoma 2 (Bcl-2) down-regulation did not occur and EGCs apoptosis was reduced. Bcl-2 overexpression also rescued high E2-induced EGCs from apoptosis.

Conclusions: High E2 concentrations induced EGCs apoptosis through enhancing IκB-α expression, which in turn suppressed NF-κB expression. The decreased nuclear NF-κB subsequently inhibited Bcl-2 expression and accordingly enhanced EGC apoptosis. (J Clin Endocrinol Metab 99: E971–E980, 2014)

Embryo implantation into the maternal endometrium is an indispensable step in mammalian reproduction. Successful embryo implantation requires a receptive endometrium, a functional embryo at the blastocyst stage, and a synchronized dialogue between the embryo and maternal endometrium (1). Adequate estradiol (E2) priming of the endometrium is required for endometrial growth, and the subsequent progesterone stimulation induces fa-

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Abbreviations: Bcl-2, B cell lymphoma 2; E2, estradiol; EGC, endometrial glandular cell; ER, estrogen receptor; IκB, inhibitory-κB; IKK, IκB kinase; IVF, in vitro fertilization; MPA, medroxyprogesterone acetate; NF-κB, nuclear factor-κB; PI, propidium iodide; PR, progesterone receptor; siRNA, small interfering RNA.
vorable endometrial receptivity (2). However, the optimal E₂ concentration before progesterone stimulation has not been clearly established.

In in vitro fertilization (IVF), multifollicular development is achieved by gonadotropins to increase the number of embryos available for transfer, and ovarian stimulation inevitably causes a high serum E₂ concentration. Several reports have found that high serum E₂ levels result in lower pregnancy and embryo implantation rates (3–5), although others did not show this cause-and-effect relationship (6–8). The underlying mechanism of decreased embryo implantation potential caused by high E₂ concentrations remains unidentified. Although the deleterious effects on embryos or oocytes have been reported (3), more researchers advocate that the detrimental effects of high E₂ concentrations arise from the impaired endometrial receptivity (4, 5, 9–11).

Basir et al (12) reported that high estrogen led to delayed glandular maturation and advanced stromal morphology, and this gland-stromal dyssynchrony might result in deficient secretory transformation of endometrium that represented a suboptimal endometrial environment for embryo implantation. Liu et al (11) found that a high serum E₂ concentration affected gene expression profiles of human endometrium at the periimplantation stage. Aberrant Dickkopf 1, a Wnt-signaling molecule, might account for the impaired embryo attachment and implantation in vivo. Altered expression of steroid receptors was also detected in endometrial cells after the treatment with high E₂ concentrations (9). However, these mechanisms are not fully clarified and deserve further investigation.

Decreased apoptosis has been found in endometrial glandular and stromal cells in women with endometriosis (13). In addition, apoptosis generally occurs in human endometrium through late secretory to menstruating phases. It appears that apoptosis is a common biological phenomenon occurring in endometrial cells. However, effects of high E₂ concentrations on endometrial cell apoptosis have never been studied. Because endometrial glandular cells (EGCs) are the cells that embryos first interact with during the implantation process (2), we try to examine in vitro EGC apoptosis under the effects of high E₂ concentrations in this study.

Materials and Methods

The experimental design of this study started with the effects of different concentrations and durations of E₂ treatment on EGC apoptosis, followed by the verification of EGC apoptosis with various experimental methods including apoptotic bodies, DNA fragmentation analysis, and cell cycle and apoptosis determination. After that, the mechanism of high E₂-induced EGC apoptosis was examined with Western blotting, promoter reporter assay, cell cycle analysis, small interfering RNA (siRNA) strategy, and gene overexpression.

EGC purification

This study consisted of six women who had endometrial polyps and underwent hysteroscopic polypectomy. All of them were in the follicular phase at premenopausal status. Spare endometrial tissue was collected for experimental use. Informed consent was obtained from each woman before surgery, and this study protocol was approved by the institutional review board at our hospital. EGC purification was done according to the method described before (14). The EGCs purity was approximately 90%, which was assessed by flow cytometry using antibodies against cytokeratin 8 (Santa Cruz Biotechnology), EGCs obtained from each woman were pooled together for the following experiments.

Reagents and antibodies

E₂ was acquired from Sigma. Antibodies to cytochrome c (sc-13561), estrogen receptor (ER)-α (sc-543), progesterone receptor (PR) (sc-7208), IκB kinase (IKK)-α (sc-7606), IKK-β (sc-7329), IKK-γ (sc-8330), inhibitory-κB (IκB)-α (sc-203), nuclear factor-κB (NF-κB) p65 (sc-372), B cell lymphoma 2 (Bcl-2; sc-509), and B-actin (sc-47778) were obtained from Santa Cruz Biotechnology. ICI 182 780 was purchased from Tocris Bioscience.

In vitro embryo implantation assay

Human BeWo choriocarcinoma cells (American Type Culture Collection; CCL-98) were plated onto noncoated plastic petri dishes at 2 × 10⁵ cells/mL in full growth medium. Abundant trophoblast spheroids formed spontaneously by cell aggregation after 24 hours of culture. Those with a diameter between 100 and 200 μm were picked and labeled with 10 μM CellTracker Green (Invitrogen Co). They served as a substitute of blastocysts in the following experiment.

In vitro implantation assay was done according to a previous report (15). Briefly, EGCs were pretreated with 10⁻⁷ M medroxyprogesterone acetate (MPA) together with different concentrations of E₂ for 48 hours. The labeled spheroids were added to EGC monolayers grown in 96-well at approximately 100 cells/mL in full growth medium. Abundant trophoblast spheroids formed spontaneously by cell aggregation after 24 hours of culture. Those with a diameter between 100 and 200 μm were picked and labeled with 10 μM CellTracker Green. They served as a substitute of blastocysts in the following experiment.

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Apoptotic bodies examined by fluorescence microscopy

EGCs were fixed with 1% formaldehyde in PBS on ice and then treated with ribonuclease A (Sigma) 10 μg/mL for 10 minutes at room temperature. Cells were subsequently stained with Hoechst 33258 (Molecular Probes Inc) 5 μg/mL. Fluorescent...
H33258-stained nuclei were digitally photographed under a fluorescence microscope (Olympus).

DNA fragmentation analysis

DNA was extracted from EGCs with the phenol-chloroform method. Electrophoresis on 2% agarose gels in a Tris/acetate acid/EDTA buffer was performed. The gels were stained with ethidium bromide to demonstrate the DNA fragments.

Cell cycle and apoptosis determination by flow cytometry

EGCs were stained with propidium iodide (PI), and cell cycle analysis was done by quantification of DNA content with FAC-Scan and Cell Quest software (Becton Dickinson Immunocytometry Systems).

Western blotting

Cytoplasmic and nuclear proteins of EGCs were extracted by cytoplasmic and nuclear extraction kits (Thermo Scientific) and were quantified by Bio-Rad protein assay (Bio-Rad Laboratories). A 20-µg protein sample was separated by SDS-PAGE, transferred onto a polyvinylidene difluoride membrane, and immunoblotted with various antibodies.

Promoter construction and reporter assay

NF-κB binding site-driven luciferase plasmids (BD Bioscience) were imported into EGCs in six-well plates using the TransFast transfection reagent (Promega Co). Twenty-four hours after transfection, EGCs were serum starved for 24 hours and then treated with different E2 concentrations (0, 10⁻⁷, 10⁻⁵, 10⁻⁴ M). To control the transfection efficiency, cells were cotransfected with pSV-β-galactosidase.

siRNA-transfected EGCs

Optimal siRNAs were purchased from Santa Cruz Biotechnology. The targeted siRNA for IκB-α and control siRNA were sc-29360 and sc-37007, respectively. Cultured EGCs were transfected with siRNA using the siRNA transfection protocol provided by Santa Cruz Biotechnology.

Bcl-2 overexpression

pWPT constructs (Addgene plasmid 12255) were described and provided by Dr Trono (16). The Bcl-2 cDNA fragment was generated by PCR, and human cancer cell line cDNA served as the template. Primers were designed according to Bcl-2 mRNA (NM_000633.2) as follows: forward primer, 5'-GTCGTGAGCGCGATCTCCTTTCCCTCTGGAGATT-3', and reverse primer, 5'-TTCCCTCGAGGTCGACATGTTGACCTCAGTTGG-3'. Two-step PCR conditions were initiated with a denaturing step at 95°C for 2 minutes, followed by five cycles of 95°C for 30 seconds and 68°C for 1 minute 30 seconds. After that, 27 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute were done and were concluded with an interval of 72°C for 8 minutes. The PCR products were purified and cloned between BamHI and SalI of pWPT lentiviral expression vector by an In-Fusion cloning kit (CLONTECH Laboratories Inc).

Lentiviral production was done as described previously (17).

Briefly, 293T cells were cotransfected with 12.5 µg of pWPT-Aggat-3' and reverse primer, 5'-TTCCCTCGAGGTCGACATGTTGACCTCAGTTGG-3'. Two-step PCR conditions were initiated with a denaturing step at 95°C for 2 minutes, followed by five cycles of 95°C for 30 seconds and 68°C for 1 minute 30 seconds. After that, 27 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute were done and were concluded with an interval of 72°C for 8 minutes. The PCR products were purified and cloned between BamHI and SalI of pWPT lentiviral expression vector by an In-Fusion cloning kit (CLONTECH Laboratories Inc).

Lentiviral production was done as described previously (17).

Briefly, 293T cells were cotransfected with 12.5 µg of pWPT-
Bcl-2, 3.2 μg pMD2.G, and 9.3 μg psPAX2 constructs using calcium phosphate. The virus-containing supernatants were harvested 48 hours after transfection. For transduction, EGCs were seeded on six-well plates and spin infected with lentiviral particles in the presence of 8 μg/mL polybrene at 1000 × g for 40 minutes.

### Statistical analysis
In this study, each experiment was repeated at least three times on different occasions. Data were presented as mean ± SE. The data were examined with one-way ANOVA, followed by Tukey test for multiple comparisons. Significance level was set as \( P < .05 \) by a two-tailed test. Statistical Program for Social Sciences (SPSS version 12; SPSS Inc) was used for calculation.

### Results

#### EGC apoptosis induced by different concentrations and durations of E2
Cytochrome c is a component of the electron transport chain in mitochondria and is often released from mitochondria during the early stage of apoptosis. Compared with cytochrome c expression after the treatment with \( 10^{-7} \) M MPA together with \( 10^{-9} \) and \( 10^{-8} \) M E2 for 6 days, there was a slight increase of cytochrome c expression after stimulation with \( 10^{-5} \) M E2 treatment for 2 days. Moreover, a significant increase of cytochrome c expression was found after stimulation with \( 10^{-7} \) M E2 for 6 days and \( 10^{-4} \) M E2 treatment for 2 days (Figure 1B). It appeared that an extremely high concentration (\( 10^{-5} \) and \( 10^{-4} \) M) of E2 treatment for 2 days achieved a similar apoptotic effect on EGCs to that caused by \( 10^{-7} \) M E2 for 6 days.

#### Estrogen and progesterone receptors
After EGCs were treated with \( 10^{-7} \) M MPA and different concentrations (0 to \( 10^{-4} \) M) of E2 for 48 hours, Western blot did not reveal obvious change of ER-\( \alpha \) and PR-A and PR-B in EGCs (Figure 1C).

#### In vitro embryo implantation assay
In vitro BeWo spheroid-EGC implantation assay demonstrated that treatment of EGCs with \( 10^{-9} \) and \( 10^{-7} \) M E2 for 48 hours increased the embryo implantation rates gradually, as compared with that without E2 treatment. However, the stimulation with high E2 concentrations (\( 10^{-5} \) and \( 10^{-4} \) M) decreased the embryo implantation potentials (Figure 1E). There was a bell-shape dose-response curve of E2 in the possibilities of embryo implantation.

#### High E2 concentrations induced EGC apoptosis
To evaluate the E2 effects, EGCs were treated with different E2 concentrations (0, \( 10^{-7}, 10^{-5}, 10^{-4} \) M) in vitro for 48 hours. Apoptotic bodies were visible in EGCs treated with \( 10^{-5} \) and \( 10^{-4} \) M E2 but not in those stimulated with 0 and \( 10^{-7} \) M E2 (Figure 2A). The addition of ER antagonist ICI 182 780 reversed the high-concentration, E2-induced DNA laddering (Figure 2B).

The EGC apoptosis was also examined with flow cytometry to detect the proportion of cells with subdiploid DNA content (Figure 2C). This is a method to detect cells losing some of their DNA in the late-stage apoptotic process. Quantitative results revealed that high E2 concentrations (\( 10^{-5} \) and \( 10^{-4} \) M), as compared with 0 and \( 10^{-7} \) M, significantly increased the percentage of sub-G1 phase. Addition of ICI 182 780 reduced the EGC apoptosis treated with \( 10^{-4} \) M E2 (Figure 2D). These results demonstrated EGC apoptosis after the stimulation with supraphysiologically high E2 concentrations.

#### High E2 concentrations enhanced IkB-\( \alpha \) expression in EGCs
To evaluate E2 effects on NF-\( \kappa \)B related protein expression, EGCs were stimulated with different E2 concentrations for 24 hours. The level of IkB-\( \alpha \) was increased after high E2 concentrations (\( 10^{-5} \) and \( 10^{-4} \) M) treatment, and levels of IKK (IKKa, IKKB, IKKy) were not changed. Meanwhile, the nuclear NF-\( \kappa \)B p65 was reduced after high concentration E2 treatment (Figure 3A). To confirm the E2 effects on NF-\( \kappa \)B activity, EGCs were pretransfected with NF-\( \kappa \)B binding site-driven luciferase plasmid prior to E2 treatment. It revealed that luciferase activity was significantly decreased in high E2-treated EGCs (Figure 3B). These results demonstrated that high E2 concentrations enhanced IkB-\( \alpha \) protein expression, which in turn inhibited NF-\( \kappa \)B activity in EGCs.

#### IkB-\( \alpha \) plays an important role in high E2-induced EGCs apoptosis
To examine the role of IkB-\( \alpha \) in high E2-induced EGC apoptosis, EGCs were pretreated with either IkB-\( \alpha \) siRNA or control siRNA for 24 hours, followed by stimulation with \( 10^{-4} \) M E2 for 48 hours. EGC apoptosis was evaluated by PI staining and flow cytometry analysis (Figure 4A). Quantitative results revealed that pretreatment of IkB-\( \alpha \) siRNA, but not control siRNA, significantly decreased the percentage of sub-G1 phase (\( P = .001 \), Figure 4B). This result suggests that IkB-\( \alpha \) plays an important role in high E2-induced EGC apoptosis.

#### IkB-\( \alpha \) suppressed Bcl-2 expression after high E2 stimulation
Bcl-2 is an important antiapoptosis protein expressed in human endometrium. We examined E2 effects on Bcl-2 expression in EGCs and found that treatment with...
Figure 2. High E2 concentrations induced EGC apoptosis. A, Apoptotic bodies were detected by Hoechst 33258 staining (arrow) in EGCs cultured with high-concentration E2 (10^{-5} and 10^{-4} M) but not in those treated with 0 and 10^{-7} M E2. B, Total genomic DNA was isolated from EGCs for DNA laddering assay. DNA fragmentation was visible after separation by gel electrophoresis in EGCs stimulated with 10^{-5} M E2 and is more prominent when EGCs were cultured with 10^{-4} M E2. After the addition of ICI 182 780 in 10^{-4} M E2-treated EGCs, the DNA laddering disappeared. C, Cell cycle was determined by PI staining and flow cytometry analysis. D, Quantitative results revealed that high E2 concentrations (10^{-5} and 10^{-4} M), as compared with 10^{-7} and 0 M, significantly increased the percentage of sub-G1 phase of EGCs, ie, cell apoptosis. The addition of ICI 182 780 decreased cell apoptosis in EGCs treated with 10^{-4} M E2 (n = 3). *, P < .001 as compared with groups of 0 M, 10^{-7} M, and 10^{-4} M E2 with ICI 182 80.
high E2 concentrations decreased Bcl-2 mRNA expression (Figure 5A) and protein level (Figure 5B). To determine the relationship between IκB-α and Bcl-2, EGCs were pretreated with IκB-α siRNA or control siRNA for 24 hours prior to E2 stimulation. Results revealed that the high E2-induced Bcl-2 down-regulation did not occur when EGCs were pretreated with IκB-α siRNA (Figure 5C). It means that IκB-α is an upstream protein that suppressed Bcl-2 expression after high concentration E2 stimulation.

**Bcl-2 overexpression rescued high E2-induced EGCs apoptosis**

Bcl-2 overexpression strategy was used to verify the role of Bcl-2 in high-concentration E2-induced EGC apoptosis. EGCs were infected with Bcl-2 overexpression vector, and the expression level of Bcl-2 protein was determined. An increased Bcl-2 protein level was detected 8 hours after Bcl-2 overexpression, and reached a maximum at 48 hours (Figure 5D). Bcl-2 overexpressed EGCs were stimulated with 10⁻⁴ M E₂ thereafter for 48 hours, and EGC apoptosis was determined by flow cytometry analysis (Figure 5E). Quantitative results revealed that Bcl-2 overexpression significantly reduced the percentage of sub-G₁ phase of EGCs treated with 10⁻⁴ M E₂ (P < .001, Figure 5F). It implies that Bcl-2 plays an important role in the high E2-induced EGC apoptosis pathway.

Taken together, our results revealed that high E2 concentrations induced EGC apoptosis through IκB-α-dependent Bcl-2 down-regulation pathway (Figure 6).
Discussion

EGCs are the cells that embryos first interact with during the implantation process. This study revealed that the impaired endometrial receptivity after high E₂ stimulation was probably due to EGCs apoptosis, which was verified by the appearance of apoptotic bodies, the presence of DNA laddering, and an increased percentage of sub-G₁ phases in the cell cycle (Figure 2). Nevertheless, cross-reactivity of E₂ to bind to other nuclear receptors might occur when the E₂ concentration is high.

An in vitro E₂ concentration of 10⁻⁹ to 10⁻⁸ M is generally regarded as a physiological concentration (19), and the one higher than that is a supraphysiological concentration. Our results revealed that stimulation with 10⁻⁵ and 10⁻⁴ M E₂ for 2 days achieved a similar apoptotic effect on EGCs to that produced by 10⁻⁷ M E₂ treatment for 6 days (Figure 1B). The in vitro embryo implantation model generally requires approximately 7 days of E₂/progestogen stimulation for endometrial cells to create an optimal environment for embryo implantation (20, 21). However, primary culture of human cells might lose their
phenotype approximately 3 days later (22). Accordingly, the stimulation with $10^{-5}$ and $10^{-4}$ M E$_2$ for 2 days was used in this study to replace the effect of $10^{-7}$ M E$_2$ treatment for 6 days and to represent the periimplantation high estrogen effects. In addition, the treatment with $10^{-7}$ M E$_2$ for 2 days was regarded as the physiological E$_2$ concentration.

Endonuclease activation is a characteristic feature of apoptosis that degrades genomic DNA and produces DNA fragments. DNA laddering assay is commonly used to detect apoptotic DNA fragmentation (23). With this method, we found that DNA fragmentation increased after EGCs were cultured with $10^{-4}$ M E$_2$, and the addition of ER antagonist ICI 182 780 reversed the high concentration E$_2$ induced DNA laddering (Figure 2B). It implies that the high concentration E$_2$ induced EGCs apoptosis is through the binding of E$_2$ with ER.

Estrogen has multiple physiological actions, including antiinflammatory, vasodilatory, apoptotic (24, 25), and antiapoptotic (26–28) effects. E$_2$ plays an important role in the regulation of endometrial cell apoptosis during an ovarian cycle. The expression of proliferating cell nuclear antigen (a proliferation marker) is higher in the follicular phase when endometrial cells are under direct effects of E$_2$, whereas endometrial cell apoptosis is intense at the mid-luteal phase when the E$_2$ effect is diminished (29).

NF-$\kappa$B is an important transcription factor for apoptosis. Our results revealed that high E$_2$ concentrations induced EGCs apoptosis through the down-regulation of NF-$\kappa$B. Cell apoptosis is a well-known NF-$\kappa$B mediated phenomenon (30), and E$_2$ has been found to interact with NF-$\kappa$B and modulate its activity (31). The E$_2$-induced NF-$\kappa$B down-regulation was also found in human macrophages (32) at an in vitro concentration of $10^{-7}$ M. In another report, a $10^{-8}$ M E$_2$ concentration enhanced NF-$\kappa$B activation and induced Jurkat cell (immortalized T cell) apoptosis but decreased the apoptosis of human peripheral blood T cells (30). It appears that E$_2$ has inconsistent effects on NF-$\kappa$B expression in different cells, and various E$_2$ concentrations probably have diverse effects on the expression of NF-$\kappa$B.

In the resting status, NF-$\kappa$B exists in the cytosol as an inactive form and is associated with its inhibitory protein I$\kappa$B-$\alpha$ (33, 34). When an extracellular stimulus induces phosphorylation and subsequent degradation of I$\kappa$B-$\alpha$, the active complex of NF-$\kappa$B translocates from cytosol into nucleus and triggers various inflammatory responses (35). Our results revealed that I$\kappa$B-$\alpha$ was enhanced after high E$_2$ stimulation and accordingly decreased the NF-$\kappa$B nuclear translocation (N-p65) in EGCs (Figure 3). This finding is consistent with previous studies, in which E$_2$ suppresses NF-$\kappa$B activation through the inhibition of I$\kappa$B-$\alpha$ phosphorylation in human macrophages (32) and rat aortic smooth muscle cells (36). The treatment of EGCs with I$\kappa$B-$\alpha$ siRNA prior to high E$_2$ stimulation decreased EGCs apoptosis (Figure 4B). This finding further verified the role that I$\kappa$B-$\alpha$ played in the high E$_2$-induced EGCs apoptosis.

Bcl-2 mediates antiapoptotic signals in a wide variety of human cells. Our results revealed that Bcl-2 expression was suppressed after the treatment with high E$_2$ concentrations (Figure 5, A and B), and the overexpression of Bcl-2 rescued EGCs from apoptosis (Figure 5F). The treatment of EGCs with I$\kappa$B-$\alpha$ siRNA prior to high E$_2$ stimulation increased Bcl-2 expression (Figure 5C), suggesting that Bcl-2 was a downstream protein of I$\kappa$B-$\alpha$ in the high E$_2$-induced EGCs apoptosis signaling pathway.

Biological relevance of the findings in this study is that the most beneficial E$_2$ concentration for embryo implantation is within a limited range. Although the deficiency of E$_2$ might not be favorable, extreme high levels of E$_2$ are also detrimental to embryo implantation. This finding is consistent with the bell-shape dose response curve of E$_2$. Nevertheless, the high E$_2$-induced EGCs apoptosis is probably a temporary rather than a permanent effect. In hyperstimulated IVF cycles with a serum E$_2$ level greater than 3000–5000 pg/mL and/or the number of retrieved oocytes more than 15–20, cryopreservation of all the embryos is an effective method to prevent the occurrence of ovarian hyperstimulation syndrome. Transferring these frozen-thawed embryos in subsequent cycles has been found to achieve higher pregnancy and embryo implantation rates than transferring fresh embryos in the hyperstimulated cycles (37–39). It implies that endometrial receptivity is impaired under the direct effect of high E$_2$ concentrations, and it recovers when the high E$_2$ effect is gone.

In conclusion, in vitro stimulation of high E$_2$ concentrations ($10^{-5}$ and $10^{-4}$ M) enhanced I$\kappa$B-$\alpha$ expression,
which in turn inhibited NF-κB expression. The decreased expression of NF-κB subsequently suppressed Bcl-2 protein and accordingly increased EGCs apoptosis. This high E2-induced EGCs apoptosis might account for the defective endometrial receptivity in women with excessively high E2 concentrations after ovarian hyperstimulation in IVF cycles.

Acknowledgments

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