

## Original Article

# Ovarian IL-1 $\alpha$ and IL-1 $\beta$ levels are associated with primary ovarian insufficiency

Huan Yang<sup>1</sup>, Hui Pang<sup>2</sup>, Congxiu Miao<sup>3</sup>

<sup>1</sup>Changzhi Medical College, Changzhi, China; <sup>2</sup>Department of Immunology, Changzhi Medical College, Changzhi, China; <sup>3</sup>Department of Reproductive Genetics, Heping Hospital of Changzhi Medical College, Institute of Reproduction and Genetics, Changzhi Medical College, Changzhi, China

Received June 13, 2018; Accepted July 19, 2018; Epub September 1, 2018; Published September 15, 2018

**Abstract:** Primary ovarian insufficiency (POI) is characterized by premature ovarian failure and lack of menstrual periods in women under the age of 40. Ample evidence suggests that interleukin 1 (IL-1) family proteins are involved in the processes of ovarian follicular development and depletion. The association of these cytokines with POI disorder and the underlying molecular mechanism in regulation of the ovarian functions remain largely undetermined. In this study, follicular fluids and serum samples were harvested from POI patients and healthy women who underwent follicular aspiration during *in vitro* fertilization. Enzyme-linked immunosorbent assay (ELISA) and the quantitative real-time PCR (qPCR) were employed to assess the levels of protein secretion and mRNA expression of IL-1 $\alpha$  and IL-1 $\beta$ , as well as the molecules that are critical for apoptotic pathways. Our results showed that IL-1 $\alpha$  level in serum samples and follicular fluid of POI patients was significantly elevated in comparison to that in healthy women. Interestingly, the follicular levels of both IL-1 $\alpha$  and IL-1 $\beta$  and TNF- $\alpha$  were significantly higher than their serum levels. Our qPCR analysis further revealed that there was a significant upregulation of apoptotic Bax mRNA expression, but expression of anti-apoptotic factor Bcl-2 mRNA expression was downregulated in POI patients. In conclusion, our studies revealed that the elevated level of IL-1 in follicle fluids of POI patients may be the major causal factor for follicular apoptosis that consequently impairs follicle reserve and ovarian functions by follicle depletion.

**Keywords:** Primary ovarian insufficiency, IL-1, follicle fluids, apoptosis

## Introduction

Primary ovarian insufficiency (POI) is the clinical condition that is characterized by the absence of menstrual periods (amenorrhea) before the age of 40. It is primarily due to premature follicular cell depletion that consequently leads to a diminished ovarian reserve (DOR) and women's infertility [1-3].

Early studies have shown that apoptosis plays essential roles in ovarian follicle reservation and atretic follicle formation. 99% of follicles appear to degenerate throughout a woman's life. Apoptosis is a process of programmed cell death that is governed by pro- and anti-apoptotic regulators, such as BAX, BCL-2, TNF- $\alpha$ , and the Fas/Fas ligand (Fas/FasL) system. Early studies have shown that there are more primary follicles in adult ovaries lacking of Bax protein [4]. Bcl-2, on the other hand, reduces follicular atresia by inhibiting apoptosis of gr-

anulosa cells [5]. The Fas/FasL system has been shown to be one of the key mediators of apoptosis in atretic follicles. It was demonstrated that Fas/FasL is involved in p53-mediated apoptosis in granulosa cells during follicular development and atresia [6-8]. Interestingly, TNF- $\alpha$  level is negatively associated with the quality of oocytes [9]. Hence, the delicate balance between these critical molecules determines the fate of follicular cells to survival or death, and disruption of this balance will adversely influence the ovarian functions and ultimately affect women's reproductive physiology [10].

Interleukin-1 (IL-1) family proteins are critical cytokines in regulation of inflammation, innate immunity, angiogenesis, and hematopoiesis [11]. IL-1 $\alpha$  and IL-1 $\beta$  are the most studied members that regulate proliferation and apoptosis in a variety of cell types [11]. Early studies showed that IL-1 levels were closely associated with

gonadotropin levels and pregnancy rates [12]. Patients with higher level of IL-1 $\beta$  exhibited a higher successful rate of pregnancy after embryo transplantation [12]. IL-1 $\beta$  has been shown to play an important role in follicle maturation and ovulation in mice [13] and rabbits [14]. By employing the genetic mutation mouse models, recent studies by Shiri Uri-Belapolsky et al [15] demonstrated that the capacity of ovarian reserve improved greatly in absence of IL-1 $\alpha$  expression. These results indicate that IL-1  $\alpha$  plays a deteriorative role, but IL-1 $\beta$  plays a favorable role in follicle maturation and ovarian functions.

IL-1 $\alpha$  and IL-1 $\beta$  are present in the serum and follicular fluid of patients who underwent IVF-ET. The clinical association between IL-1 production and POI condition remains unclear. The mechanism underlying the elevated level of IL-1 in regulation of human ovarian function requires further investigation.

### Materials and methods

#### *Recruitment criteria*

The sample collection protocols were approved by the Human Reproduction Committee of the University, Changzhi Medical College. From May 2016 to September 2016, 42 patients who were undergoing IVF-ET at the Reproductive Medicine Center of Heping Hospital affiliated with Changzhi Medical College and Reproductive Medicine Centre of Maternal and Child Health Hospital of Shanxi province enrolled in this study. 22 patients who underwent IVF-ET due to male infertility were recruited as the control group. The POI group included 17 patients with DOR and 3 patients with POF. The DORs referred to hypomenorrhea, oligomenorrhea, amenorrhea, and infertility, and meet the following basal serum hormone criteria when diagnosed with transvaginal ultrasonography during the early follicular phase: follicle-stimulating hormone (FSH), 10~40 IU/L (2 intervals for more than one month); FSH/lutropin (LH), > 3.6 [16, 17]; estradiol (E2), > 85 pg/ml [18], MOD (mean ovarian diameter), < 20 mm; and AFC (antral follicle count)  $\leq$  5 [19]. POFs with amenorrhea last for at least 4 months with perimenopausal symptoms and meet following basal serum hormone criteria: FSH: > 40 IU/L (2 intervals for more than one month) for 2 or more times; E2 < 20~30 pg/ml; and AMH (anti-Mullerian hormone): < 1.26 ng/ml [12]. All patients exhibited normal liver and kidney func-

tion without serious infection. All patients were informed of the study and provided consent.

#### *Specimen collection*

Fasting peripheral venous blood samples were obtained from the control group on days 2 to 4 of the menstrual period, and at any time from the POI patient group (no menstrual cycle). All the samples included one coagulant and one anticoagulant preparation. The coagulation sample was immediately centrifuged at 3000 rpm for 20 min and the supernatant was dispensed into 200  $\mu$ l centrifuge tubes. The anticoagulation sample was immediately dispensed into 1 ml centrifuge tubes without RNase. Both samples were stored at -80°C.

After successful induction of ovulation, patients underwent follicular aspiration by transvaginal B-scan ultrasound-guided puncture of the posterior fornix to collect ootids on the day of ovulation. Follicular fluid was obtained from the follicular puncture (> 16 mm diameter), and the cumulus complex was identified and separated. The autologous follicular fluids (red blood cells < 10<sup>4</sup>/ml) were pooled from each patient, and the follicular fluid was centrifuged at 3000 rpm for 10 minutes. The supernatant was harvested, aliquoted, and stored at -80°C until the assays were performed.

#### *Enzyme-linked immunosorbent assay*

Follicular fluid and serum samples from patients were tested for IL-1 $\alpha$  and IL-1 $\beta$ . The ELISA kits were used to quantitatively determine the levels of IL-1 $\alpha$  (Abcam, Human Interleukin-1 $\alpha$  ELISA kit, ab100560, British) and IL-1 $\beta$  (Abcam, Human Interleukin-1 $\beta$  ELISA kit, ab100562, British) according to the manufacturer's specifications. Quantitation was performed with use of the standard dose-dependent curve, and the cytokine concentrations detected in the samples are given in pg/mL. The intra-assay and interassay coefficients of variation were < 10% and < 12% for the IL-1 $\alpha$  and IL-1 $\beta$  assay, respectively. The detection limits were 0.5 pg/mL for IL-1 $\alpha$  and 0.3 pg/mL for IL-1 $\beta$ .

#### *RNA isolation and reverse transcription*

Total RNA was isolated from peripheral venous blood samples which were stored at -80°C in 1 ml centrifuge tubes without RNase using Omega Blood RNA Kit (r6814-01) according to the manufacturer's instructions and was quantified with the Microplate reader (TECAN Infinite

## IL-1 in primary ovarian insufficiency

**Table 1.** Comparison of basic data between the POI group and the control group ( $\bar{x} \pm SD$ )

Group	Cases	Ages	BMI (kg/m <sup>2</sup> )	FSH (mIU/ml)	FSH/LH
POI group	20	32.49 ± 3.93	21.56 ± 3.97	22.06 ± 9.83	4.11 ± 1.51
Control group	22	30.91 ± 4.78	21.33 ± 4.05	6.99 ± 1.54	1.87 ± 0.71
<i>P</i> value	-	> 0.05	> 0.05	< 0.001*	< 0.001*

Note: \*,  $P < 0.05$ .

**Table 2.** Comparison of IL-1 $\alpha$  and IL-1 $\beta$  between the POI group and the control group ( $\bar{x} \pm SD$ )

Item (pg/ml)	POI group (20)	Control group (22)	<i>P</i> value
Serum IL-1 $\alpha$	1.07 ± 1.61	0.48 ± 0.53	0.025*
Follicular fluid IL-1 $\alpha$	3.65 ± 4.16	1.67 ± 1.03	< 0.001*
Serum IL-1 $\beta$	1.45 ± 1.84	1.17 ± 1.62	0.771
Follicular fluid IL-1 $\beta$	3.11 ± 3.06	1.66 ± 1.12	0.054

Note: \*,  $P < 0.05$ .

**Table 3.** Comparison of IL-1 $\alpha$  in serum and follicular fluid within each group

Item	Serum IL-1 (pg/ml)	Follicular fluid IL-1 $\alpha$ (pg/ml)	<i>P</i> value
POI group	1.07 ± 1.61	3.65 ± 4.16	< 0.001*
Control group	0.48 ± 0.53	1.67 ± 1.03	< 0.001*

Note: \*,  $P < 0.05$ .

**Table 4.** Comparison of IL-1 $\beta$  in serum and follicular fluid within each group

Item	Serum IL-1 $\beta$ (pg/ml)	Follicular fluid IL-1 $\beta$ (pg/ml)	<i>P</i> value
POI group	1.45 ± 1.84	3.11 ± 3.06	< 0.001*
Control group	1.17 ± 1.62	1.66 ± 1.12	0.009*

Note: \*,  $P < 0.05$ .

M200 PRO NanoQuant). First-strand cDNA was synthesized by PCR Gene Amplification Instrument (Thermo Scientific) from a total of 4  $\mu$ g of RNA using 1  $\mu$ L of reverse transcriptase, incubating 15 minutes at 42°C and heating 5 seconds at 85°C.

### Quantitative real-time PCR

cDNA was amplified (40 cycles) with 10  $\mu$ M gene-specific primers (Supplementary Table 1) using Fluorescence Quantitative PCR (Applied Biosystems 7500 Real-Time PCR System). Changes in the level of mRNA expression were detected using gene-specific primers and fast SYBR green master mix reagent (TransGen Biotech Easy Taq DNA Polymerase kit, AP111-11 and Qiagen Quanti Fast SYBR Green PCR Kit). The thermal cycling conditions for the Fast

SYBR Green reaction were 5 minutes at 95°C, followed by 40 cycles of 5 seconds at 95°C and 30 seconds at 60°C. PCR primers were designed by Primer 5.0 software and synthesized by Invitrogen Biotechnology Co., Ltd.

### Statistical analysis

SPSS 17.0 software was used for statistical analysis. Data were shown as the mean  $\pm$  standard deviation ( $\bar{x} \pm SD$ ). The Student *t* test was used as applicable. The Mann-Whitney test was used to assess differences for the means. The association among IL-1 $\alpha$ , Bax, Bcl-2, Fas, FasL, and TNF- $\alpha$  was used as continuous variables on the Pearson correlation test. The criterion was set as  $\alpha = 0.05$ .  $P < 0.05$  was considered significant. All experiments were repeated at least three times and had similar results.

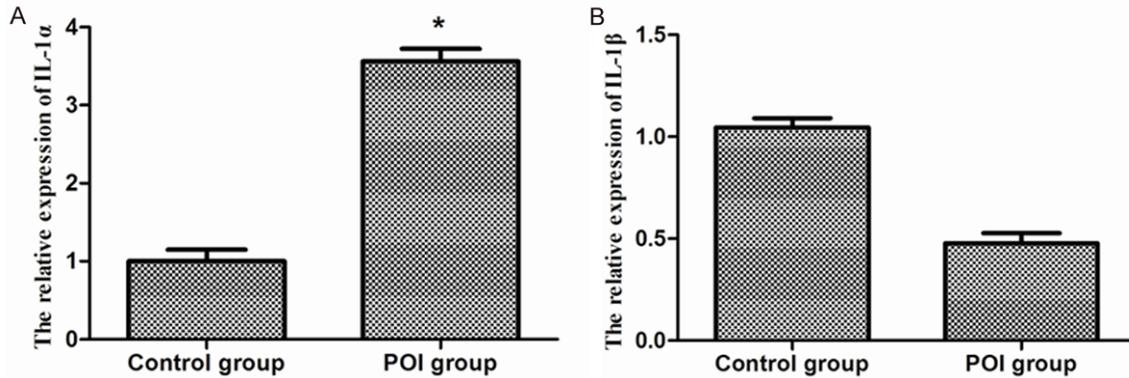
### Results

We first compared the basic data of POI group with the control group. As shown in **Table 1**, no significant difference was observed in the ages and BMIs between these two groups ( $P > 0.05$ ). Interestingly, there was a significant increase in the levels of FSH and FSH/LH in the POI group when compared to controls ( $P < 0.001$ ).

We next determined the levels of IL-1 in the serum and follicular fluid samples collected from these two groups. Our results showed that the IL-1 $\alpha$  level in POI group was significantly higher compared to that in control group ( $P = 0.025$ ,  $P < 0.001$ ). There was no difference in the levels of IL-1 $\beta$  in these samples ( $P = 0.771$ ,  $P = 0.054$ ) (**Table 2**).

We also compared IL-1 levels between serum and follicular fluid of POI patients and healthy

## IL-1 in primary ovarian insufficiency



**Figure 1.** A. Comparison of mRNA expression levels of IL-1 $\alpha$  between the two groups. B. Comparison of mRNA expression levels of IL-1 $\beta$  between the two groups. \*,  $P < 0.05$ .

women. Both IL-1 $\alpha$  and IL-1 $\beta$  levels in follicular fluid were significantly higher than those observed in their serum levels ( $P < 0.001$ ). The analyzed data for IL-1 $\alpha$  and IL-1 $\beta$  levels in serum and follicular fluid samples harvested from the control and POI groups are summarized in **Tables 3** and **4**, respectively.

To further confirm the levels of IL-1 $\alpha$  and IL-1 $\beta$  in POI patients, quantitative real-time PCR was employed to assess gene expression of these two cytokines. Our results revealed there was a significant increase in the levels of IL-1 $\alpha$  mRNA expression in POI group in comparison to the control group (**Figure 1A**,  $P < 0.05$ ), whereas IL-1 $\beta$  mRNA expression of remained unchanged (**Figure 1B**,  $P > 0.05$ ).

Interestingly, mRNA expression levels of Bax and TNF- $\alpha$  were significantly higher in the POI group (**Figure 2A-C**,  $P < 0.05$ ), whereas the level of Bcl-2 mRNA expression was reduced significantly. No significant difference in the levels of Fas and FasL mRNA expression was observed (**Figure 2D, 2E**,  $P > 0.05$ ).

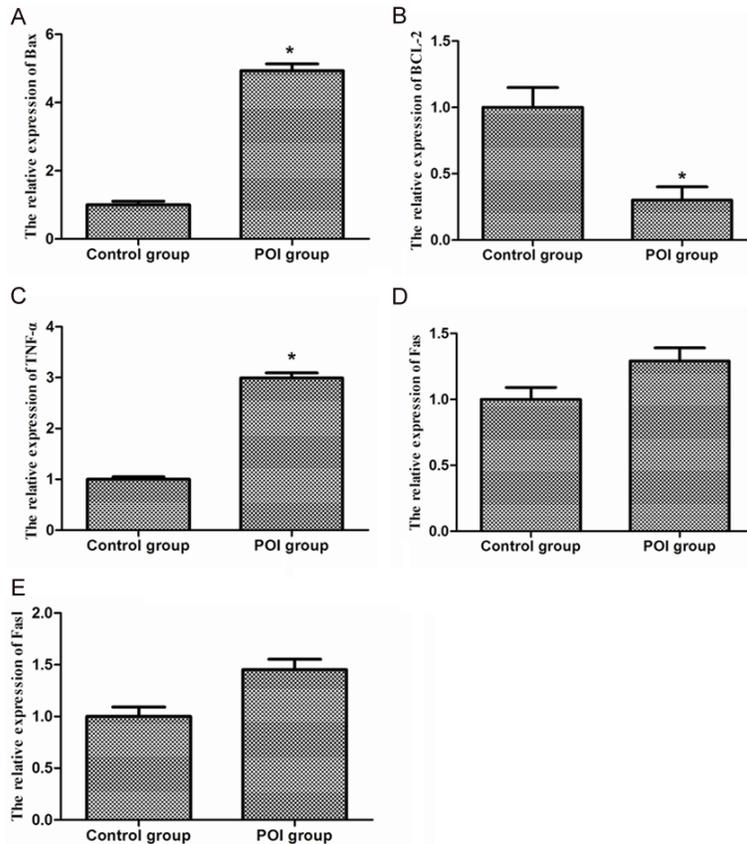
### Discussion

Early studies have shown that the proinflammatory cytokines IL-1 $\alpha$  and IL-1 $\beta$  in follicle fluids are synthesized in ovarian follicle granulosa cells and oocytes. In this study, our results showed that these cytokines were elevated significantly in follicular fluids and serum samples of POI patients. Recent studies by Shiri Uri-Belapolsky showed that IL-1 $\alpha$ -knockout female mice exhibited higher pregnancy rates and larger litter sizes in comparison to WT control

females at advanced ages. In addition, the number of secondary and antral follicles and the level of serum anti-Müllerian hormone, a putative marker of ovarian reserve, was markedly higher in these mutants [15]. On the other hand, IL-1 $\beta$ -KO mice displayed a comparable but slightly longer ovarian lifespan [15]. It is plausible to envisage that the elevated level of IL-1 in follicle fluids of POI patients may elicit inflammation and impair ovarian functions by accelerating depletion of ovarian reserves.

Apoptosis is a process of programmed cell death that occurs in multicellular organisms, including the developing ovarian follicles. It is tightly controlled by apoptotic or antiapoptotic factors, such as BCL-2 family proteins. Bcl-2 is the founding member of the Bcl-2 family that regulate by either inducing (pro-apoptotic) or inhibiting (anti-apoptotic) apoptosis. Apoptosis regulator BAX (known as bcl-2-like protein 4) is also a member of the Bcl-2 gene family that forms a heterodimer with BCL2 and functions as an apoptotic activator [20]. Bax mediates the release of cytochrome C from mitochondria, which ultimately activates caspase and leads to cell death [10]. Ample evidence suggests that BCL-2 family proteins are critically involved in follicular maturation and atresia [4]. Loss of Bax expression led to a reduction in the average litter size and the number of oocytes ovulated in response to exogenous gonadotropin stimulation. These females also exhibited longer ovarian life in mice at old age [5, 8, 21, 22]. Early studies have shown that the Bcl-2/Bax ratio controls ovarian cell fate determination of ovarian granulosa cells to apoptotic pathways [22]. In chemotherapy-

## IL-1 in primary ovarian insufficiency



**Figure 2.** A. Comparison of mRNA expression levels of Bax between the control group and the POI group. B. Comparison of mRNA expression levels of Bcl-2 between the control group and the POI group. C. Comparison of mRNA expression levels of TNF- $\alpha$  between the control group and the POI group. D. Comparison of mRNA expression levels of Fas between the control group and the POI group. E. Comparison of mRNA expression levels of FasL between the control group and the POI group. \*,  $P < 0.05$ .

induced POI mouse model, ovarian damage was associated with an increased level of Bax expression and decreased level of Bcl-2 expression [23, 24]. Interestingly, our current study showed that mRNA expression levels of the proapoptotic factors Bax and TNF- $\alpha$  were significantly higher in POI patients, whereas the antiapoptotic factor Bcl-2 mRNA level was concomitantly reduced in these samples. Early studies indicate that IL-1 family proteins are involved in apoptotic pathways in many cell types by affecting BAX and Bcl-2 expression [25, 26]. Hence, we postulate that IL-1-induced inflammation may promote ovarian follicle cell apoptosis by disturbing the BAX/BCL-2 balance. In support of this notion, we observed an increased level of TNF- $\alpha$  in POI patients. TNF- $\alpha$  is involved in apoptosis by means of the glutathione signaling pathway, ceramide signaling pathway, and calcium release in many cell

types including ovarian follicular cells [27, 28]. TNF- $\alpha$  regulates the process of follicular atresia by inducing the apoptosis of oocytes, stromal cells, and granulosa cells. In addition, TNF- $\alpha$  level in follicular fluid negatively correlated with embryo quality and pregnancy rate [29]. The number of apoptotic granulosa cells is closely associated with the serum levels of pro-inflammatory cytokines IL-6, IL-8, and TNF- $\alpha$  in chemotherapy-induced ovarian failure [30]. The apoptotic role of TNF- $\alpha$  in ovarian follicle cells of POI patients is under investigation in our laboratory.

The Fas/FasL system also plays an important role in regulation of granulosa cell apoptosis during the process of ovarian follicle atresia [6, 31]. However, we did not observe any statistically significant changes in the Fas/FasL system between POI patients and healthy women. It remains elusive whether species heterogeneity contributes to this discrepancy.

Early studies showed that the IL-1 $\beta$  level in follicular fluid was positively correlated with the number of mature follicles and the number of eggs obtained [32]. IL-1 $\beta$ -KO female mice displayed a comparable but more subtle prolongation of ovarian lifespan [15]. In this study, the follicular IL-1 $\beta$  level in POI patients was higher than that in healthy women, but not statistically significant. The role of follicular IL-1 $\beta$  on ovarian function in POI patient needs to be further investigated.

Our results also revealed that the levels of IL-1 $\alpha$  and IL-1 $\beta$  in follicular fluids were significantly higher than those in serum. These results are consistent with the notion of "IL-1 ovarian origin" of these cytokines. Using western blot analysis and immunohistochemistry technology, Uri-Belapolsky [15] showed that IL-1 $\alpha$  and IL-1 $\beta$  are detected in granulosa cells and oocyte

cytoplasm throughout follicular development [33, 34]. It remains intriguing to identify the causal factors that induce production IL-1 $\alpha$  and IL-1 $\beta$  in ovarian follicles of POI patients [35].

In summary, our studies for the first time provided the clinical evidence that IL-1 levels in follicular fluids and serial samples of POI patients are significant higher than healthy women. We propose a mechanism by which IL-1  $\alpha$ -mediated inflammatory response promotes ovarian follicle apoptosis and adversely impact ovarian functions in POI patients. Our studies clearly indicate that IL-1 $\alpha$  emerges as a new diagnostic and therapeutic target for POI and POI-associated disorders.

### Acknowledgements

This study was supported by a research project from Shanxi Provincial Health and Family Planning Commission (No.2015157) and Doctorpreneurs Start-up funding from Changzhi Medical College (No.BS15011). The author(s) would like to thank Dr. Xueqing Wu (Maternal and Child Health Hospital in Taiyuan) for the specimens used in this study and Dan Li and Min Li for technical assistance.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Congxiu Miao, Department of Reproductive Genetics, Heping Hospital of Changzhi Medical College, Institute of Reproduction and Genetics, Changzhi Medical College, Changzhi 046000, China. Tel: 86-355-3128552; E-mail: mcxms@163.com

### References

- [1] Rafique S, Sterling EW, Nelson LM. A new approach to primary ovarian insufficiency. *Obstet Gynecol Clin N Am* 2012; 39: 567-86.
- [2] De Vos M, Devroey P, Fauser BC. Primary ovarian insufficiency. *Lancet* 2010; 376: 911-21.
- [3] Practice committee of the american society for reproductive medicine. testing and interpreting measures of ovarian reserve: a committee opinion. *Fertil Steril* 2015; 103: e9-e17.
- [4] Perez GI, Robles R, Knudson CM, Flaws JA, Korsmeyer SJ, Tilly JL. Prolongation of ovarian lifespan into advanced chronological age by bax-deficiency. *Nat Genet* 1999; 21: 200-3.
- [5] Greenfeld CR, Babus JK, Furth PA, Marion S, Hoyer PB, Flaws JA. BAX is involved in regulating follicular growth, but is dispensable for follicle atresia in adult mouse ovaries. *Reproduction* 2007; 133: 107-16.
- [6] Chen Q, Yano T, Matsumi H, Osuga Y, Yano N, Xu J, Wada O, Koga K, Fujiwara T, Kugu K, Taketani Y. Cross-Talk between Fas/Fas ligand system and nitric oxide in the pathway subserving granulosa cell apoptosis: a possible regulatory mechanism for ovarian follicle atresia. *Endocrinology* 2005; 146: 808-15.
- [7] Lin PF, Hao YB, Guo HL, Liu HL, Rui R. Role of Fas/FasL on apoptosis of porcine follicular granulosa cells derived from isolated follicles during culture in vitro. *Dongwuxue Yanjiu* 2010; 31: 268-74.
- [8] Inoue N, Matsuda F, Goto Y, Manabe N. Role of cell-death ligand-receptor system of granulosa cells in selective follicular atresia in porcine ovary. *J Reprod Dev* 2011; 57: 169-75.
- [9] Lee KS, Joo BS, Na YJ, Yoon MS, Choi OH, Kim WW. Relationships between concentrations of tumor necrosis factor-alpha and nitric oxide in follicular fluid and oocyte quality. *J Assist Reprod Genet* 2000; 17: 222-8.
- [10] Hussein MR. Apoptosis in the ovary: molecular mechanisms. *Hum Reprod Update* 2005; 11: 162-77.
- [11] Ganaie M, AbuElhija M, Lunenfeld E, Cherniy N, Weisze N, Itach SB, Breitbart H, Apte R, Huleihel M. Effect of interleukin-1 receptor antagonist gene deletion on male mouse fertility. *Endocrinology* 2009; 150: 295-303.
- [12] Karagouni EE, Chryssikopoulos A, Mantzavinou T, Kanakas N, Dotsi EN. Interleukin-1beta and interleukin-1alpha may affect the implantation rate of patients undergoing in vitro fertilization-embryo transfer. *Fertil Steril* 1998; 70: 553-9.
- [13] Brännström M, Wang L, Norman RJ. Ovulatory effect of interleukin-1 beta on the perfused rat ovary. *Endocrinology* 1993; 132: 399-404.
- [14] Takehara Y, Dharmarajan AM, Kaufman G, Wallach EE. Effect of interleukin-1 beta on ovulation in the in vitro perfused rabbit ovary. *Endocrinology* 1994; 134: 1788-93.
- [15] Uri-Belapolsky S, Shaish A, Eliyahu E, Grossman H, Levi M, Chuderland D, Ninio-Many L, Hasky N, Shashar D, Almog T, Kandel-Kfir M, Harats D, Shalgi R, Kamari Y. Interleukin-1 deficiency prolongs ovarian lifespan in mice. *Proc Natl Acad Sci U S A* 2014; 111: 124927.
- [16] Mukherjee T, Copperman AB, Lapinski R, Sandler B, Bustillo M, Grunfeld L. An elevated day three follicle-stimulating hormone: luteinizing hormone ratio (FSH: LH) in the presence of a normal day 3 FSH predicts a poor response

## IL-1 in primary ovarian insufficiency

- to controlled ovarian hyperstimulation. *Fertil Steril* 1996; 65: 588-93.
- [17] Chae HD, Kim CH, Kang BM, Chang YS. Clinical usefulness of basal FSH as a prognostic factor in patients undergoing intracytoplasmic sperm injection. *J Obstet gynecol Res* 2000; 26: 55-60.
- [18] Kwee J, Schats R, McDonnell J, Lambalk CB, Schoemaker J. Intercycle variability of ovarian reserve tests: results of a prospective randomized study. *Hum Reprod* 2004; 19: 590-5.
- [19] Bancsi LF, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. Impact of repeated antral follicle counts on the prediction of poor ovarian response in women undergoing in vitro fertilization. *Fertil Steril* 2004; 81: 35-41.
- [20] Hussein MR, Haemel AK, Wood GS. Apoptosis and melanoma: molecular mechanisms. *J Pathol* 2003; 199: 275-88.
- [21] Bao L, Zhang SF, Zhang YP. Research progress of premature ovarian failure. *International Journal of Reproductive Health/Family Planning* 2011; 30: 479-83.
- [22] He X, Wang SY, Yin CH, Wang T, Jia CW, Ma YM. Hydrogen-rich water exerting a protective effect on ovarian reserve function in a mouse model of immune premature ovarian failure induced by zona pellucida 3. *Chin Med J (Engl)* 2016; 129: 2331-7.
- [23] Ling L, Feng X, Wei T, Wang Y, Wang Y, Zhang W, He L, Wang Z, Zeng Q, Xiong Z. Effects of low-intensity pulsed ultrasound (LIPUS)-pretreated human amnion-derived mesenchymal stem cell (hAD-MSC) transplantation on primary ovarian insufficiency in rats. *Stem Cell Res Ther* 2017; 8: 283.
- [24] Sun L, Li D, Song K, Wei J, Yao S, Li Z, Su X, Ju X, Chao L, Deng X, Kong B, Li L. Exosomes derived from human umbilical cord mesenchymal stem cells protect against cisplatin-induced ovarian granulosa cell stress and apoptosis in vitro. *Sci Rep* 2017; 7: 2552.
- [25] Simonart T, Van Vooren JP. Interleukin-1 beta increases the BCL-2/BAX ratio in kaposi's sarcoma cells. *Cytokine* 2002; 19: 259-66.
- [26] Mahr S, Neumayer N, Gerhard M, Classen M, Prinz C. IL-1beta-induced apoptosis in rat gastric enterochromaffin-like cells is mediated by iNOS, NF-kappaB, and Bax protein. *Gastroenterology* 2000; 118: 515-24.
- [27] Watson RW, Rotstein OD, Parodo J, Bitar R, Marshall JC. The IL-1 $\beta$ -Converting enzyme (Caspase-1) inhibits apoptosis of inflammatory neutrophils through activation of IL-1 $\beta$ . *J Immunol* 1998; 161: 957-62.
- [28] Kaipia A, Chun SY, Eisenhauer K, Hsueh AJ. Tumor necrosis factor-alpha and its second messenger, ceramide, stimulate apoptosis in cultured ovarian follicles. *Endocrinology* 1996; 137: 4864-70.
- [29] Fabian D, Juhás S, Il'ková G, Koppel J. Dose- and time-dependent effects of TNFalpha and actinomycin D on cell death incidence and embryo growth in mouse blastocysts. *Zygote* 2007; 15: 241-9.
- [30] Luo Q, Yin N, Zhang L, Yuan W, Zhao W, Luan X, Zhang H. Role of SDF-1/CXCR4 and cytokines in the development of ovary injury in chemotherapy drug induced premature ovarian failure mice. *Life Sci* 2017; 179: 103-9.
- [31] Mori T, Xu JP, Mori E, Sato E, Saito S, Guo MW. Expression of Fas-Fas ligand system associated with atresia through apoptosis in murine ovary. *Horm Res* 1997; 48 Suppl 3: 11-9.
- [32] Wang W, Shen HM, Xu SX, et al. Changes of interleukin -1 beta in peripheral blood and follicular fluid during controlled ovarian hyperstimulation cycles. *Journal of Reproductive Medicine* 2001; 10.
- [33] Florio P, Rossi M, Viganò P, Luisi S, Torricelli M, Torres PB, Di Blasio AM, Petraglia F. Interleukin 1 beta and progesterone stimulate activin a expression and secretion from cultured human endometrial stromal cells. *Reprod Sci* 2007; 14: 29-36.
- [34] de los Santos MJ, Anderson DJ, Racowsky C, Simón C, Hill JA. Expression of interleukin-1 system genes in human gametes. *Biol Reprod* 1998; 59: 1419-24.
- [35] Wang XF, Xing FQ, Chen SL. Interleukin-1beta expression on ovarian granulosa cells and its clinical implication in women undergoing in vitro fertilization. *Di Yi Jun Yi Da Xue Xue Bao* 2002; 22: 934-6.

## IL-1 in primary ovarian insufficiency

### **Supplementary Table 1.** Specific primers used in tests

---

#### Human- $\beta$ -actin

Forward primer: TGGCACCCAGCACAAATGAA

Reverse primer: CTAAGTCATAGCCGCCTAGAA

#### IL-1alpha

Forward primer: AATGACGCCCTCAATCAAAGTA

Reverse primer: CTTTCATCTTGGGCAGTCACATA

#### IL-1beta

Forward primer: ATCTGTACCTGTCCTGCGTGTT

Reverse primer: ATCCTGGCCGCCTTTGGTCCCT

#### Bax

Forward primer: GAGGAACTGGACAGTAACATGGAGCT

Reverse primer: CGGCCCCAGTTGAAGTTGC

#### Bcl-2

Forward primer: ATTCCTGCATCTCATGCCAAGGG

Reverse primer: TGTGCTTGCATTCTTGGACGAGG

#### TNF-alpha

Forward primer: TGTAGCCCATGTTGTAGCAAACC

Reverse primer: GAGGACCTGGGAGTAGATGAGGTA

#### Fas

Forward primer: CCTGCCAAGAAGGGAAGGAG

Reverse primer: TTTGGTGCAAGGGTCACAGT

#### FasI

Forward primer: AAGAGAGGGAACCACAGCAC

Reverse primer: CATCTGGCTGGTAGACTCTCG

---