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**Original Research Article**

# Effect of Yushen zhuyun decoction on rats with diminished ovarian reserve induced by tripterygium glycosides

**Yuli Jin[1], Ying Zhang[2], Lishuang Hao[3], Li Tan[4], Mengfei Zhuang[4] and Tingting Zhang[4]***

*[1]Department of Traditional Chinese Medicine, [2]Department of Endocrinology, Shanghai General Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200080, [3]Department of Gynecology, Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200082, [4]Department of Gynecology, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200437, China*

**For correspondence: **Email:** ttzhangyy@sina.com; **Tel:** +86 21-65161782*

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## Abstract

***Purpose:*** *To investigate the effect of Yushen zhuyun decoction (YSZYF) on rats with diminished ovarian reserve (DOR).*
***Methods:*** *High-performance liquid chromatography (HPLC) was used to determine the major phytochemical constituents of YSZYF. Rats with DOR (DOR rats) were prepared by administration of tripterygium glycosides (TWP) orally (50 mg/kg) for 15 days. Thereafter, DOR rats were treated orally with YSZYF (300, 600 and 900 mg/kg). After 15 days' treatment, ovary index was calculated and blood was obtained to determine serum levels of follicle-stimulating hormone (FSH), estradiol (E2), progesterone (P), testosterone (T), inhibin (INH) and anti-mullerian hormone (AMH) by radioimmunoassay and enzyme-linked immunosorbent assay (ELISA). In addition, the ovary was subjected to histopathological examinations.*
***Results:*** *Phytochemical investigation indicated that the major constituents of YSZYF are acteoside, loganin, lcariin and echinacoside. Compared to the control rats, YSZYF treatment enhanced the ovary index of DOR rat ($p < 0.05$); furthermore, YSZYF treatment also enhanced the number of follicles and corpus luteum, as well as alleviated inflammatory reaction in ovary tissues. Additionally, the serum levels of FSH and T were elevated by treatment of YSZYF ($p < 0.01$), whereas E2, INHB and AMH concentrations decreased ($p < 0.01$), compared to control rats.*
***Conclusion:*** *The findings indicate that YSZYF improves ovarian reserve of DOR rats, and thus has a potential for treating infertility.*

***Keywords:*** *Yushen zhuyun decoction, Diminished ovarian reserve, Infertility, Acteoside, Loganin, Lcariin, Echinacoside*

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## INTRODUCTION

Infertility is a worldwide reproductive health problem which could destroy the happiness in individuals and families [1,2]. Currently, it is reported that *in vitro* fertilization and embryo transfer (IVF-ET) could improve the pregnancy rate of husband and wife with infertility; however, the pregnancy rate is still less than 50% [3,4]. The reasons for infertility are complex, and the diminished ovarian reserve (DOR) is one of the most important reasons [5,6]. Ovarian reserve (OR) is defined as the number and quality of remaining oocytes in ovarian cortex, reflects the

fertility potency and reproductive endocrine function of women [7,8]. DOR indicates that the follicle can be recruited and the egg productive abilities of ovaries are diminished, leading to a decrease in a woman's fertility [7,9].

Traditional Chinese Medicine (TCM) has been used in China for treating various complicated diseases, and are also considered a potential approach for solving infertility [10,11]. *Yushen zhuyun* decoction (YSZYF, Table 1) is an effective clinical TCM for treating infertility in China [12,13]. However, so far, experimental research on YSZYF regarding its detail phytochemical and pharmacological activity is still lacking. Consequently, this study was aimed to investigate the major chemical composition of YSZYF, and explore its possible pharmacological mechanisms in infertility.

## EXPERIMENTAL

### Chemicals and reagents

Tripterygium glycosides (TWP) tablets (10 mg/tablet) were purchased from Jiangsu MEITONG Pharmaceutical Co. Ltd. (Taizhou, China). Estradiol valerate (EV) tablets (1 mg/tablet) were purchased from the Delpharm Lille S.A.S (France); follicle-stimulating hormone (FSH), estradiol ($E_2$), progesterone (P) and testosterone (T) radioimmunoassay kits were the products of the Beijing North Institute of Biotech. (Beijing, China); Inhibin (INH) and anti-mullerian hormone (AMH) ELISA kits were purchased from the Wuhan ColorfulGene Biol Tech. Co. (Wuhan, China).

### Animals

Experimental groups consisted of Sprague Dawley (SD) rats (250 - 280 g) which were supplied by the Shanghai SLRC Lab. Animal Co. (Shanghai, China). The experimental protocols obey the international animal experimental guideline [14] and were approved by the Animal Care and Use Committee of Shanghai General Hospital (no. 15030001).

### Preparation of water extracts of YSZYF

For clinical use, YSZYF is traditionally prepared by decocting with water directly. Thus, all the crude herbal drugs of YSZYF (Table 1) were powdered and then decocted thrice with 10 times its weight of water. The mixture was filtered and the clear supernatant was concentrated *in vacuum* at 50 °C; the dry yield of YSZYF was 12.58 %.

### Phytochemical investigation of YSZYF by HPLC

The water extract of YSZYF was dissolved in methanol for preparing and testing samples for HPLC assay. HPLC assay was carried out on an Agilent Technologies 1200 system (USA) with a ZORBA× SB-$C_{18}$ column (250 × 4.6 mm, 5 µm). The mobile phase contains acetonitrile (A) and water (B) in gradient elution at a flow rate of 0.8 mL/min, 0 min A:B =10:90, after 30 min A:B 100:0. The detection wavelength was 240 nm of and the column temperature was 25 °C.

### Experimental protocols

In the present study, a total of 60 SD rats were divided into 6 groups (n=10): 1) normal rats, 2) DOR model rats, 3) positive drug treated DOR rats, and 4-6) YSZYF treated DOR rats (300, 600 and 900 mg/kg). DOR model rats were prepared by oral administration of TWP at the dose of 50 mg/kg for 15 days. Also, for the drugs treated DOR rats, EV (0.21 mg/kg) and YSZYF (300, 600 and 900 mg/kg) were also administered orally for 15 days. Furthermore, the EV and YSZYF were dissolved in water for administration. After 15 days' treatment, blood samples were collected from the abdominal aorta blood using vacuum blood collection tube under anesthesia (pentobarbital sodium, 40 mg/kg, *i.p.*). Then, rats were sacrificed with decapitation, and the ovary was separated. The weight of ovary was weight and the ovary index was calculated.

### Histopathological examination

Ovary tissues were fixed with 10 % formalin, and embedded in paraffin. The ovarian tissues were sectioned to 5 µm thickness, and stained with hematoxylin and eosin (*H&E*). The histopathological changes in ovarian tissues were examined using a microscope (Olympus, Japan).

### Determination of serum levels of FSH, $E_2$, T, P, INH and AMH

The blood samples were centrifuged (3000 rpm, 10 min) to separate serum samples, and stored at -20 °C until analysis. The serum levels of FSH, $E_2$, T and P were determined by radioimmunoassay according to the instructions of radioimmunoassay kits. In addition, the serum levels of INH and AMH were determined by ELISA method following the instructions of ELISA kits.

**Table 1:** Composition of YSZYF

| Name | Amount (g) | Name | Amount (g) |
|---|---|---|---|
| *Poria Locos* | 12 | *Rehmannia Glutinosa* | 10 |
| *Fructus Corni* | 10 | *Tortoise Plastron* | 10 |
| *Cornu Cervi Degelatinatum* | 10 | *Epimedium Herb* | 10 |
| *Radix Morindae Officinalis* | 10 | *Cistanche Deserticola* | 10 |

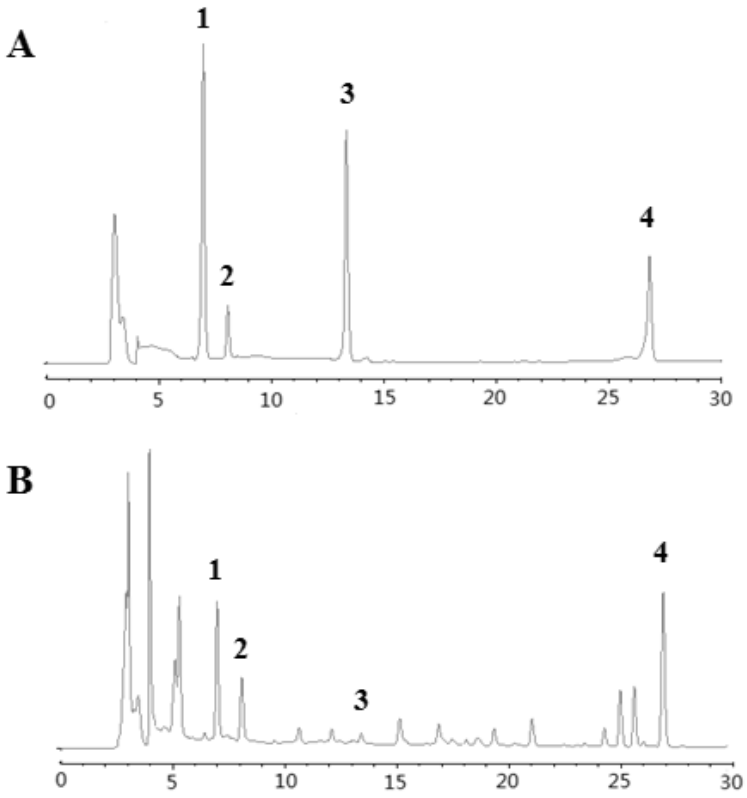


**Figure 1:** HPLC chromatogram of YSZYF. (A) HPLC spectrum of standard references compounds, **1**-**4** represented acteoside, loganin, lcariin and echinacoside, respectively; (B) HPLC spectrum of sample; The HPLC analysis was performed on an ZORBA× SB-$C_{18}$ column (250 × 4.6 mm, 5 µm). The mobile phase was composed of acetonitrile (A)-water(B) in gradient elution at a flow rate of 0.8ml/min, 0 min A:B = 10:90, after 30 min A:B 100:0. The detection wavelength was 240 nm of and the column temperature was 25 °C

### Statistical analysis

Data were presented as mean ± SD and evaluated with one-way analysis of variance (ANOVA) analysis, and the differences were considered significant at $p < 0.05$.

## RESULTS

### Phytochemical profile of YSZYF

The base peaks and UV chromatograms of the YSZYF sample by HPLC assay were presented in Figure 1. According to the results, four major phytochemical components, including acteoside, loganin, lcariin and echinacoside were detected by comparing their retention times, UV spectra with those of reference compounds.

### Ovarian index

Ovarian index data are shown in Figure 2. After administration of TWP (50 mg/kg) for 15 days, the ovarian indexes of DOR model rats were decreased obviously ($p < 0.01$), compared with the normal rats, indicating that the ovarian weights of DOR model rats decreased, compared with normal rats. Interestingly, similar to the positive drug (EV, 0.21 mg/kg), YSZYF administration at doses of 300, 600 and 900 mg/kg significantly increased ovarian indexes of DOR rats ($p < 0.05$, $p < 0.01$, $p < 0.01$), compared with DOR model rats.

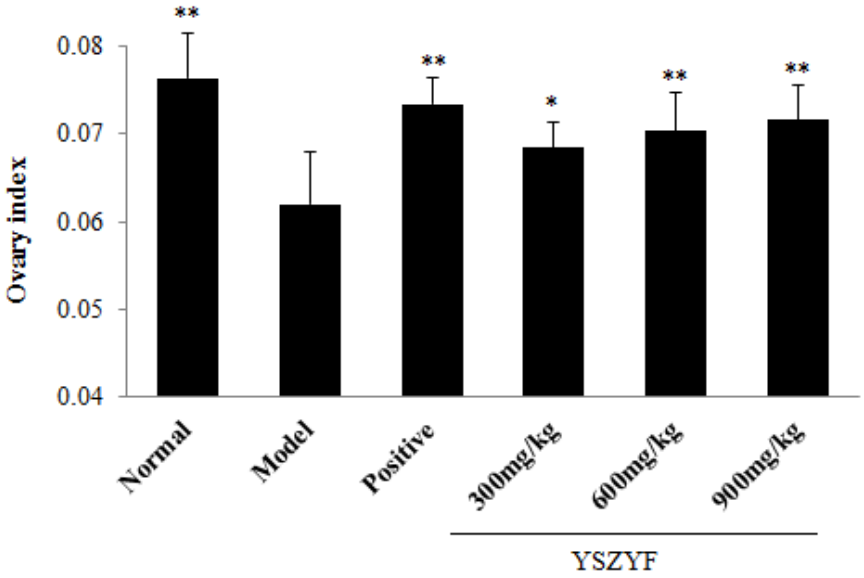


**Figure 2:** Ovarian index. Estradiol valerate was used as the positive drug, and the dose was 0.21 mg/kg. All the tested drugs were administered orally. For normal mice, normal saline was administered orally. Data are expressed as mean ± SD (n=10); *$p$ < 0.05, **$p$ < 0.01, compared with control mice

**Histopathological features**

As can be seen from Figure 3, the ovarian tissue section of normal rats showed that ovarian structure and no. of follicles are normal, while primordial follicles, primary follicles, secondary follicles and mature follicles are visible. Follicular fluid content and corpus luteum are normal, and no inflammatory response and fibrosis were observed. Furthermore, ovarian tissue sections of DOR model rats indicate inflammatory reactions, some tissues were atrophied, no. of follicles and corpus luteum decreased obviously, and structure and cell arrangement is irregular. Importantly, after treatment with EV and YSZYF, the ovarian tissue sections of DOR rats showed a significant improvement. The no. of follicles in various stages as well as the no. of corpus luteum increased, while cell arrangement and inflammatory responses improved and alleviated, respectively.

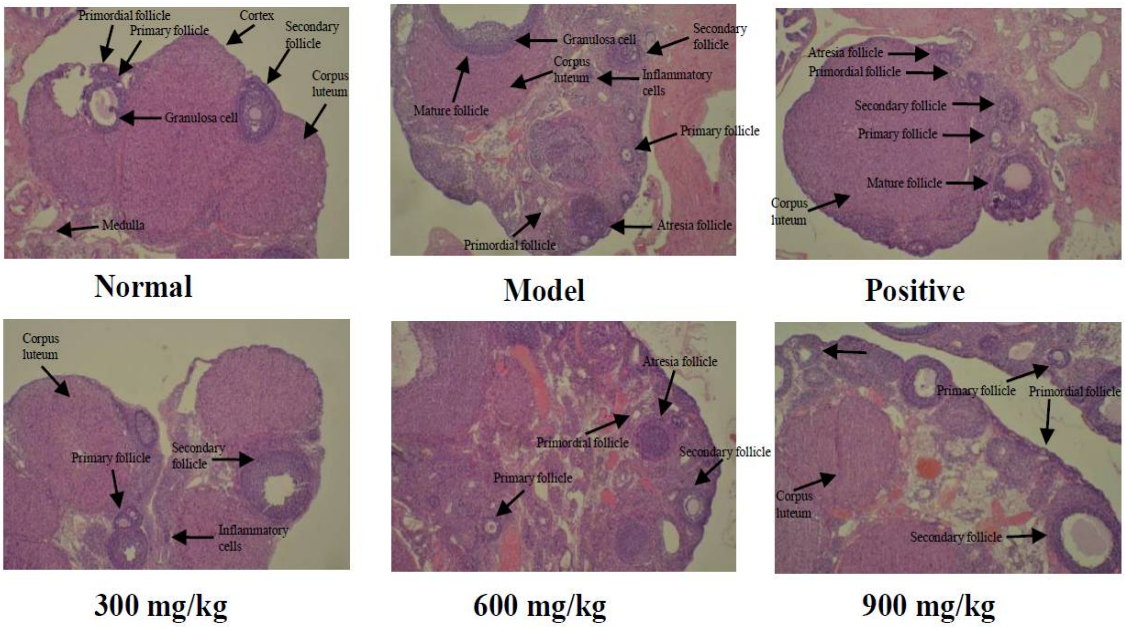


**Figure 3**: Histopathological characteristics of ovaries (× 40). Estradiol valerate (0.21 mg/kg) was used as the positive control. All the tested drugs were administered orally. For normal mice, normal saline was administered orally

**Serum levels of FSH, $E_2$, T and P**

The serum levels of FSH, $E_2$, T and P data are presented in Figure 4. After administration with TWP (50 mg/kg) for 15 days, serum levels of FSH and T significantly elevated ($p < 0.01$), compared with the normal rats. However, YSZYF treatment at doses of 300, 600 and 900 mg/kg dose-dependently decreased serum levels of FSH and T ($p < 0.01$), compared with DOR model rats. Compared with normal rats, contents of $E_2$ decreased after treating TWP (50 mg/kg) for 15 days ($p < 0.01$). In contrast, YSZYF treatment (300, 600 and 900 mg/kg) dose-dependently increased $E_2$ contents in serum ($p < 0.01$), compared with DOR model rats. Besides, no obvious change in serum level of P ($p > 0.05$) occurred.

**Effect of decoction on serum levels of INH and AMH**

Serum levels of INH and AMH are shown in Figure 5. Compared with normal rats, serum levels of INH and AMH in DOR control rats significantly decreased ($p < 0.01$). On the other, serum levels of INH and AMH increased dose-dependently following treatment with YSZYF at doses of 300, 600 and 900 mg/kg ($p < 0.01$), compared with DOR control rats.

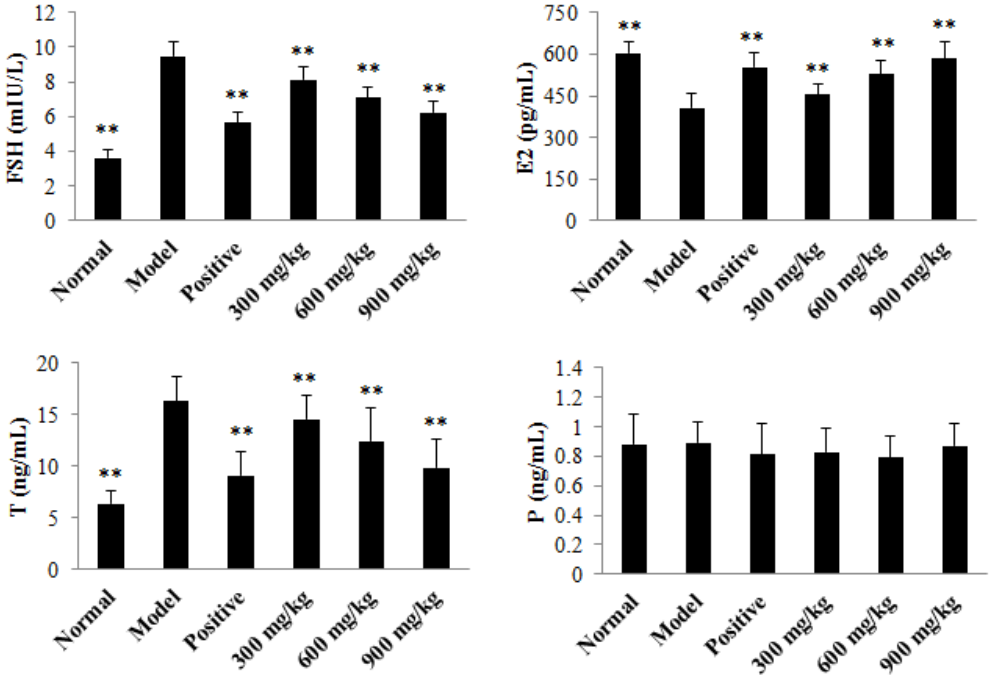


**Figure 4:** Results of FSH, $E_2$, T and P determination in serum. Estradiol valerate was used as the positive drug, and the dose was 0.21 mg/kg. All the test drugs were administered orally. For normal mice, normal saline was administered orally. Data are expressed as mean ± SD (n = 10), $^{**}p < 0.01$, compared with model mice. **FSH** = follicle-stimulating hormone, **$E_2$** = estradiol, **P** = progesterone, **T** = testosterone

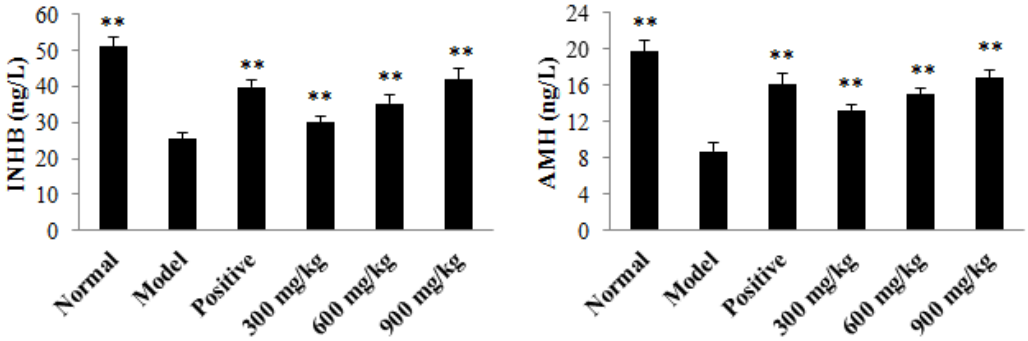


**Figure 5:** Serum levels of INH and AMH. Estradiol valerate was used as the positive drug (0.21 mg/kg). For normal mice, normal saline was administered orally. Data are expressed as mean ± SD (n=10); $^{**}p < 0.01$, compared with model mice. **INH** = inhibin, **AMH** = anti mullerian hormone

## DISCUSSION

The present work reported the effects of *Yushen zhuyun* decoction (YSZYF) on rats with diminished ovarian reserve (DOR), and revealed for the first time that YSZYF possesses significant ameliorative effects on DOR rats.

Ovarian reserve (OR) reveals the effect of the state of the ovary on egg production, and thus reflects the degree of fertility of women [1,2]. Clinically, DOR commonly reflects changes in menstrual cycle, infertility and abortion, and could decrease the success rate of *in vitro* fertilization (IVF), leading to ovarian atrophy [15]. Consequently, improving DOR is important for enhancing fertility of women. TWP-induced DOR rat model is a reliable experimental animal model to evaluate the effect of a candidate fertility drug [16]. For control rats, ovarian index and serum levels of $E_2$, INHB and AMH decreased, whereas the serum level of FSH increased compared to normal rats, indicating that DOR rats were established successfully.

The present results demonstrate that after treatment with YSZYF, ovarian index was increased, suggesting that YSZYF could be used to improve the ovarian atrophy of women with DOR. Furthermore, our study also revealed that treatment with YSZYF could increase the numbers of follicles in various stages and corpus luteums in ovary of DOR rats, indicating YSZYF can be used to improve the ovarian reserve of women with DOR. Currently, it is reported that follicular development in normal menstrual cycle dependent on the stimulation of gonadotropins, such as FSH, T, $E_2$ and P, *etc* [17].

INHB can be considered a marker of ovarian reserve, since a decrease in INHB level frequently reflects follicular decline and ovarian senescence [18,19]. AMH, released by follicular cells, is a suitable index for early ovarian reserve, and can be used to predict pregnancy outcome [20,21]. In addition, AMH is an indicator of follicle production, and usually decreases gradually with age [21]. The results indicate that serum levels of INHB and AMH increased significantly, while FSH decreased. In addition, these results indicate that YSZYF increases the follicular development and follicles number.

## CONCLUSION

The findings of this study demonstrate that YSZYF is effective in improving ovarian reservation in experimental DOR rats, and thus can enhance follicular development and follicle development. Thus, the results suggest that YSZYF possesses some potentials for treating infertility in clinical settings.

## DECLARATIONS

### *Acknowledgement*

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### *Conflict of Interest*

No conflict of interest associated with this work.

### *Contribution of Authors*

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.



## REFERENCES

1. *Ying LY, Wu LH, Loke AY. Gender differences in experiences with and adjustments to infertility: A literature review. Int J Nurs Stud 2015; 52: 1640-1652.*
2. *Cao ZY. Chinese obstetrics and gynecology. Beijing: People's Medical Publishing House, 2003; p 2323.*
3. *Chen H, Wang Y, Lyu QF, Ai A, Fu YL, Tian H, Cai RF, Hong QQ, Chen QJ, Shoham Z, Kuang YP. Comparison of live-birth defects after luteal-phase ovarian stimulation vs. conventional ovarian stimulation for in vitro fertilization and vitrified embryo transfer cycles. Fertil Steri 2015; 103: 1194-1201.*
4. *Pereira N, Gerber D, Gerber RS, Lekovich JP, Elias RT, Spandorfer SD, Rosenwaks Z. Effect of methotrexate or Salpingectomy for ectopic pregnancy on subsequent in vitro fertilization-embryotransfer outcomes. J Minim Invasive Gynecol 2015; 22: 870-876.*
5. *Reed BG, Babayev SN, Bukulmez O. Shifting paradigms in diminished ovarian reserve and advanced reproductive age in assisted reproduction: customization*

*instead of conformity. Semin Reprod Med 2015; 33: 169-178.*

6. *Boucret L, Chao de la Barca JM, Morinière C, Desquiret V, Ferré-L'Hôtellier V, Descamps P, Marcaillou C, Reynier P, Procaccio V, May-Panloup P. Relationship between diminished ovarian reserve and mitochondrial biogenesis in cumulus cells. Hum Reprod 2015; 30: 1653-1664.*
7. *Feng JH, Kong LW, Li LL. Research progress of pathogeny and treatment of traditional Chinese and Western medicine of decreasing ovarian reservation. Chin Med Hera 2014; 11: 161-164.*
8. *Roustan A, Perrin J, Debals-Gonthier M, Paulmyer-Lacroix O, Agostini A, Courbiere B. Surgical diminished ovarian reserve after endometrioma cystectomy versus idiopathic DOR: comparison of in vitro fertilization outcome. Hum Reprod 2015; 30: 840-847.*
9. *Hassa H, Aydin Y, Ozatik O, Erol K, Ozatik Y. Effects of dehydroepiandrosterone (DHEA) on follicular dynamics in a diminished ovarian reservein vivo model. Syst Biol Reprod Med 2015; 61: 117-121.*
10. *Wang Y, Fu X, Xu J, Wang Q, Kuang H. Systems pharmacology to investigate the interaction of berberine and other drugs in treating polycystic ovary syndrome. Sci Rep 2016; 6: 28089-28089.*
11. *Wu WY, Hou JJ, Long HL, Yang WZ, Liang J, Guo DA. TCM-based new drug discovery and development in China. Chin J Nat Med 2014; 12: 241-250.*
12. *Li D, Guo J. Application of kidney reinforcing and menstrual cycle regulating therapy in assisted reproductive techniques for improving ovarian reserve. J Beijing Uni TCM 2008; 31: 131-134.*
13. *Bi LJ. Clinical observation on 18 cases of hypomenorrhea treated with CAI' periodic therapy of Chinese Herbal Medicine. J Liaoning Univ TCM 2010; 12: 178-180.*
14. *U.S. Office of Science and Technology Policy. Laboratory animal welfare, U.S. government principles for the utilization and care of vertebrate animals used in testing, research and training; notice. Fed Regist 1985; 50: 20864-20865.*
15. *Tao ZE, Sui XL. The clinical significance of determination of ovarian reservation in IVF-ET. J Tianjing Med Univ 2008; 14: 561-563.*
16. *Gao H, Yang J, Han B, Xia T. Research of the animal model of premature ovarian failure. Forum TCM 2007; 22: 22-26.*
17. *Lan RX, Liu F, He ZB, Chen C, Liu SJ, Shi Y, Liu YL, Yoshimura Y, Zhang M. Immunolocalization of GnRHRI, gonadotropin receptors, PGR, and PGRMCI during follicular development in the rabbit ovary. Theriogenology 2014; 81: 1139-1147.*
18. *Xu XF, Tan Y, Xue XM, Chen XL, Yang DR, Liu Y. Clinical value of serum inhibin B in predicting ovarian function. Pro Obstet Gynecol 2008; 17: 568-570.*
19. *Dernck Rik, Zhang Y, Feng XH. Smads: transcriptional activators of TGF-beta responses. Cell 1998; 95: 737-740.*
20. *van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, Themmen AP. Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. Hum Reprod 2002; 17: 3065-3071.*
21. *Eldar-Geva T, Ben-Chetrit A, Spitz IM. Dynamic assays of inhibin B, anti-Mullerian hormone and estradiol following FSH stimulation and ovarian ultrasonography as predictors of IVF outcome. Hum Reprod 2005; 20: 3178-3183.*