Am J Transl Res 2021;13(4):3165-3173
www.ajtr.org /ISSN:1943-8141/AJTR0128638

Original Article

Sequential therapy for kidney-tonifying via traditional Chinese medicine effectively improves the reproductive potential and quality of life of women with decreased ovarian reserve: a randomized controlled study

Wenxu Duan[1], Yueyi Cheng[2]

[1]Department of Gynecology, Tianjin Nankai Hospital, Tianjin City, China; [2]Department of Surgery, Tianjin Public Security Hospital, Tianjin City, China

Received December 20, 2020; Accepted January 25, 2021; Epub April 15, 2021; Published April 30, 2021

Abstract: Objective: To investigate the effect of sequential therapy for kidney-tonifying via traditional Chinese medicine (TCM) on improving the fecundity and quality of life (QOL) of women with decreased ovarian reserve (DOR). Methods: A prospective and randomized controlled study was conducted, in which 80 infertile patients with DOR were selected in our hospital and randomly divided into the test group (n=40) and the control group (n=40). The control group was given sequential therapy of artificial menstrual cycle via administration of estrogen and progesterone, and the test group received sequential therapy for kidney-tonifying via TCM. Then, Kupperman indices, hormone levels, ovarian reserve functions, menopause specific quality of life questionnaire (MENQOL) scores, as well as the pregnancy rates within one year before and after treatment were compared between the two groups. Results: Compared with the control group, the levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and resistance index (RI) of ovarian stroma were significantly decreased in the test group (all P<0.001), while the levels of estradiol (E2), anti-Mullerian hormone (AMH), mid-luteal phase E2, progesterone, antral follicular count (AFC), and ovarian diameter (OVD) of patients were notably increased in the test group (all P<0.001). After treatment, the Kupperman indices and MENQOL scores of the test group were considerably lower than those of the control group (P<0.001). No adverse effects were observed in the test group, whereas the incidence of adverse effects in the control group was 12.50%, although without significant difference between the two groups (P>0.05). The pregnancy rate within one year in the test group was significantly higher than that of the control group (47.50% vs. 25.00%) (P<0.05). Conclusion: Sequential therapy for kidney-tonifying via TCM could effectively improve the clinical symptoms, hormone levels, and ovarian function, increase ovulation quality and pregnancy rate, and improve the QOL of DOR patients.

Keywords: Kidney-tonifying traditional Chinese medicine, decreased ovarian reserve, infertility, pregnancy, quality of life

Introduction

Decreased ovarian reserve (DOR) refers to a disease, of which the quality and quantity of oocytes are compromised before age 40 in women, leading to the decrease or loss of fertility [1]. Perimenopausal symptoms such as irregular menstruation, hot flashes and night sweats, vaginal dryness, sexual discomfort, sleep disorders, and mood swings may occur among DOR patients [2, 3]. These can result in premature ovarian failure (POF) within 1-5 years, which seriously impairs the fertility and quality of life (QOL) of women suffering from this disease [4]. Currently, the treatment of DOR is mainly by means of hormone replacement therapy (HRT) [5, 6], including estrogen and progesterone artificial menstrual cycle and ovulation induction therapies. However, HRT has been shown some caveats. For example, although the artificial menstrual cycle via estrogen and progesterone administration can quickly restore the menstrual cycle, it can't effectively improve the quality and quantity of ovulation or pregnancy rate. The DOR would reoccur after the medication withdrawal, and

Table 1. Baseline data of the two groups of patients ($\bar{x}$ ± sd)

Category	Test group (n=40)	Control group (n=40)	*P* value
Age/mean (range), year	33.4 (21.0-39.0)	32.8 (20.0-39.0)	>0.05
Disease course/mean (range), year	2.4 (0.5-4.8)	2.7 (0.4-5.5)	>0.05
FSH (U/L)	18.62±4.18	18.31±4.57	>0.05
LH (U/L)	11.62±3.39	11.54±3.24	>0.05
E2 (pmol/L)	92.57±12.28	93.18±13.47	>0.05
AMH (pmol/L)	1.33±0.24	1.36±0.28	>0.05

Note: FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: estradiol; AMH: anti-Mullerian hormone.

complications such as ovarian hyperstimulation syndrome may also be induced [7]. Therefore, it is necessary to develop a more effective and safe approach for the treatment of DOR.

Traditional Chinese medicine (TCM) is an important part of TCM-based therapeutic strategy and shows unique advantages in the treatment of many diseases. Prescriptions such as Zhuyun Tang and Guchong Tang have demonstrated excellent effects in the treatment of infertility and perimenopausal syndrome [8, 9]. Modern pharmacological studies have also validated that several TCM of kidney-tonifying and activating blood circulation play important roles in inducing ovulation and improving the balance of sex hormones. For example, Semen cuscutae [10, 11] can promote the follicle development by increasing the release of luteinizing hormone (LH) from pituitary and the response of the ovary to LH. Angelica biserrata can increase the level of deoxyribonucleic acid (DNA) in the uterus and mimic the function of the estrogen [12, 13]. Morinda officinalis also shows estrogen-like effects, which could benefit the uterine growth [14]. Recently, some researchers have adopted the theory of "preventive treatment" from TCM and demonstrated a good outcome in the treatment of DOR by kidney-tonifying and activating blood circulation in combination with hormone medications [15]. In this study, we applied two prescriptions for DOR patients via sequential treatment based on the characteristics of the menstrual cycle. Additionally, through the randomized controlled study, we elucidated the effect of sequential therapy for kidney-tonifying via TCM on the fecundity and QOL of DOR patients, which could provide more effective therapeutic strategies for the treatment of DOR.

Materials and methods

Patients

A total of 80 DOR patients in our hospital from June 2018 to December 2019 were selected in this prospective and randomized controlled study. Inclusion criteria: ① 22-40 years old; ② DOR diagnosis in accordance with the committee opinion on ovarian reserve evaluation by the American Society of Reproductive Medicine (ASRM) in 2015 [16]. Exclusion criteria: ① Patients who have received other medications within 3 months; ② Ovaries removed or patients with DOR caused by radiotherapy; ③ Organic ovarian lesion; ④ Patients who were infertile due to blocked oviduct or partners' reproductive defect. A random number table method was carried out to divide the patients into a test group (n=40) and a control group (n=40). The baseline data of the two groups was listed in **Table 1**.

Ethics statement

This study followed the Declaration of Helsinki and was approved by the Ethics Committee of our hospital. All patients were informed before the study and signed a consent form.

Treatment methods

The control group was treated by sequential therapy of artificial menstrual cycle via the administration of estrogen and progesterone: On day 5 of the menstrual cycle, estradiol valerate tablets (Bayer Healthcare Co., Ltd. Guangzhou, China, 1 mg/tablet) were orally administered, 1 mg/time, once a day, continuously for 21 days. On day 12 after taking estradiol valerate tablets, Dydrogesterone tablets (Abbott Biologicals B.V, Netherlands, 10 mg/tablet) were given, 10 mg/time, 2 times/day,

continuously for 10 days. Three menstrual cycles were considered as 1 course of treatment.

The experimental group was treated by sequential therapy for kidney-tonifying via TCM: Sequential therapy for kidney-tonifying via TCM included two prescriptions, Zhuyun Tang and Guchong Tang. On day 5 of the menstrual cycle, Zhuyun Tang was given for 10 days, while on day 15 of the menstrual cycle, Guchong Tang was given for 12 days. The components of Zhuyun Tang included Semen cuscutae 12-30 g, Fructus lycii 15 g, Fructus rubi 15 g, Morinda officinalis 12 g, Herba epimedii 10 g, Cornu cervi 10 g, Radix dipsaci 10 g, Cortex eucommiae 12 g, Mulberry 15 g, Polygonum multiflorum 10-20 g, Fluoritum 30 g, Angelica biserrata 6g. Guchong Tang were composed of Semen cuscutae 12-30 g, Fructus lycii 12-20 g, Fructus rubi 12-20 g, Morinda officinalis 12 g, Herba epimedii 10 g, Cornu cervi 10 g, Radix dipsaci 12 g, Cortex eucommiae 10 g, Eclipta prostrata 20 g, Fructus ligustri lucidi 10-20 g, Rhizoma dioscoreae 15 g. All raw materials in the prescriptions were provided by the Chinese Medicine Department of our hospital and processed into granules. The prescriptions were resolved in boiled water and taken after lunch every day, once a day. Three menstrual cycles were considered as 1 course of treatment.

Serological testing

Before treatment and after 1 course of treatment (3 menstrual cycles), the patients' serum-related hormones were tested. Five milliliters of morning fasting cubital venous blood was taken from patients, followed by centrifugation for 15 min (3000 r/min) to isolate the serum. Then ELISA was used to detect serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and Anti-Mullerian hormone (AMH). The FSH, LH, E2, and AMH kits were purchased from Shanghai Lanpai Biotechnology Co., Ltd., China, and all experiments were done according to the protocols of the kits.

Ultrasound examination

Before and after treatment, a GE730 color Doppler ultrasound diagnostic machine (GE company, United States) was utilized to detect patients' Antral follicle count (AFC), ovarian diameter (OVD), and ovarian stroma blood flow resistance index (RI). The patients were followed up for 1 year, and their pregnancy rates within 1 year were recorded.

Scale evaluation

Modified Kupperman index was applied to evaluate the clinical symptoms of patients. The total index ranged from 0 to 39. The higher the index, the more severe the patients' clinical symptoms [17].

Menopause specific quality of life questionnaire (MENQOL) was used to evaluate the QOL of patients. The total score ranged from 0 to 156. The higher the score, the worse the patients' QOL [18].

Outcome measures

Major outcome measures: ① Indices of ovarian function after treatment in the two groups, including AFC, OVD, ovarian stroma RI, mid-luteal phase E2, and progesterone levels; ② Serum hormone levels before and after treatment in the two groups, including FSH, LH, E2, and AMH; ③ The modified Kupperman indices and MENQOL scores before and after treatment in the two groups.

Secondary outcome measures: Adverse effects in the two groups and pregnancy rate within 1 year.

Statistical analysis

SPSS software version 23.0 (SPSS, Inc., Chicago, IL, USA) was used for the data statistical analysis. The counting data was expressed as the number of cases (percentage) (n, %), followed by Chi-square test with two-sided $\alpha=0.05$. For the quantitative data that followed normal distribution, the age and disease course were expressed as the mean (range). Serum hormone levels were expressed as mean ± standard deviation ($\bar{x} \pm sd$), and independent sample t test was conducted for the comparison between groups, whereas paired t test was carried out for the comparison before and after treatment within the same group with two-sided $\alpha=0.05$. $P<0.05$ indicated the statistically significant difference.

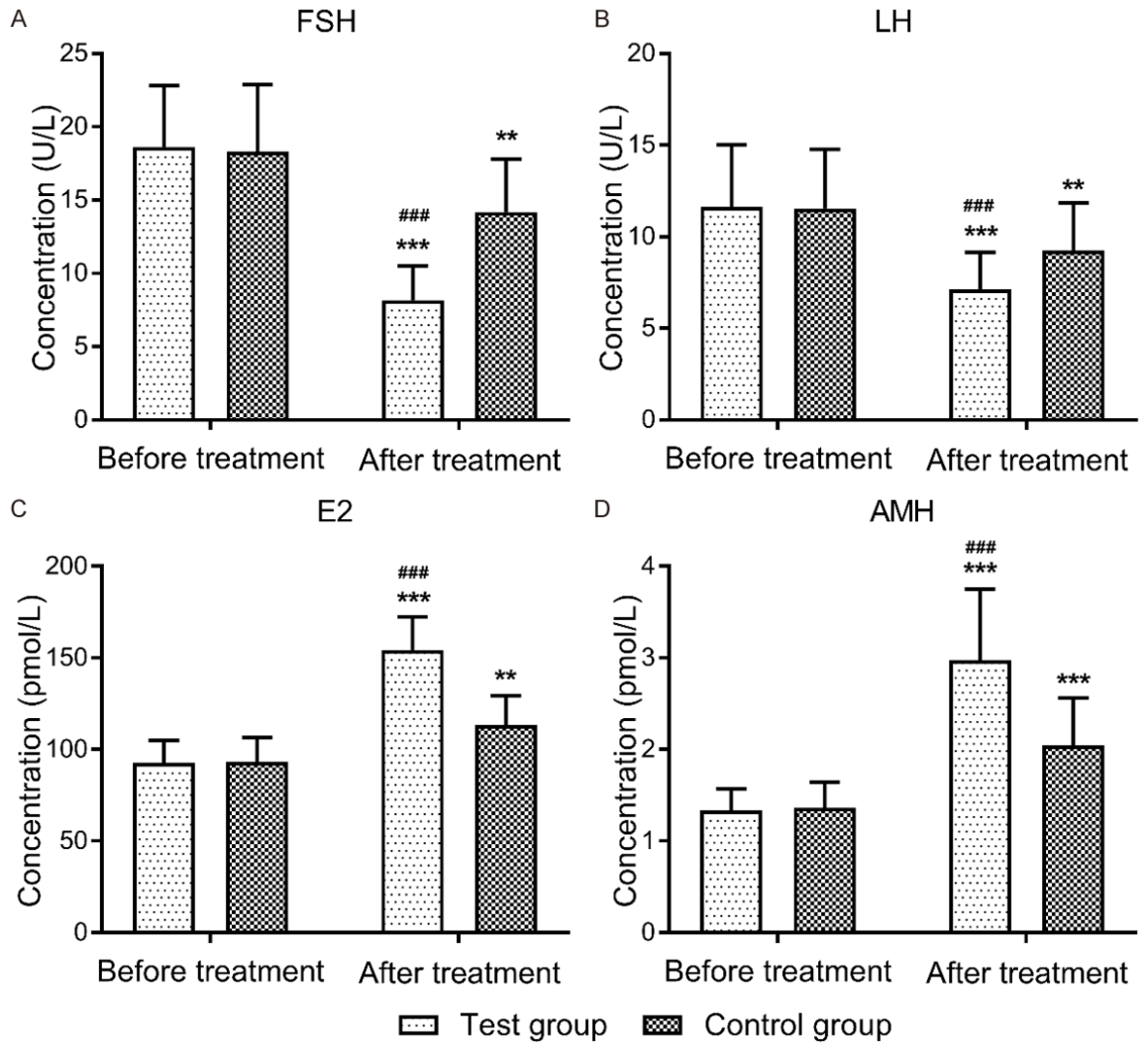

Figure 1. Hormone levels of the two groups of patients before and after treatment. A: The comparison of FSH levels between the test group and the control group before and after treatment; B: The comparison of LH levels between the test group and the control group before and after treatment; C: The comparison of E2 levels between the test group and the control group before and after treatment; D: The comparison of AMH levels between the test group and the control group before and after treatment. Compared before treatment within the group, **P<0.01, ***P<0.001; compared with the control group after treatment, $^{###}$P<0.001. FSH: follicle stimulating hormone; LH: luteinizing hormone; E2: estradiol; AMH: anti-Mullerian hormone.

Results

Baseline data

Enrolled patients were all female, 20-39 years old, with the shortest course of disease being 5 months and the longest being 5.5 years. There were no significant differences regarding the average age, average disease course, and serum hormone levels at admission between the two groups of patients (all P>0.05). See **Table 1**.

Hormone levels were more significantly improved in the test group

Before treatment, there were no significant differences in terms of the levels of FSH, LH, E2 and AMH between the two groups of patients (all P>0.05). After treatment, the levels of FSH and LH in the two groups were notably lower than those of before treatment (both P<0.01), while the levels of E2 and AMH were considerably higher than those of before treatment (both P<0.01). Additionally, compared with the control group, the levels of FSH (**Figure 1A**) and

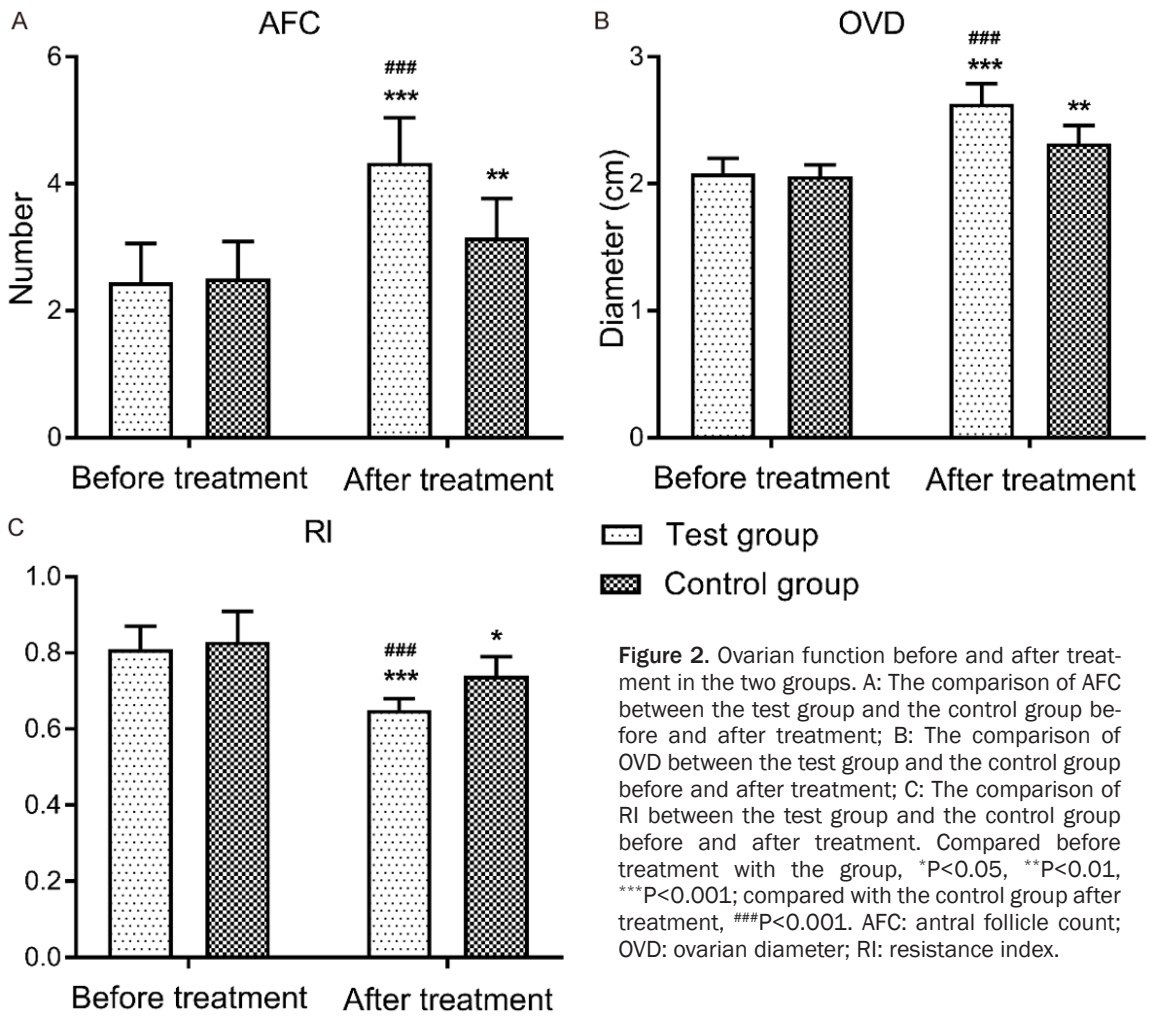

Figure 2. Ovarian function before and after treatment in the two groups. A: The comparison of AFC between the test group and the control group before and after treatment; B: The comparison of OVD between the test group and the control group before and after treatment; C: The comparison of RI between the test group and the control group before and after treatment. Compared before treatment with the group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; compared with the control group after treatment, $^{\#\#\#}P<0.001$. AFC: antral follicle count; OVD: ovarian diameter; RI: resistance index.

LH (**Figure 1B**) were significantly lower (all P<0.001), and the levels of E2 (**Figure 1C**) and AMH (**Figure 1D**) were significantly higher (all P<0.001) after treatment in the test group.

Indices of ovarian function were more significantly improved in the test group

The results of ovarian function-related indices of the two groups demonstrated that there were no significant differences in terms of AFC, OVD, and ovarian stroma RI between the two groups of patients before treatment (all P>0.05). After treatment, AFC and OVD in both groups were significantly higher than those of before treatment (both P<0.01), while ovarian stroma RI was significantly reduced (P<0.05; **Figure 2**). Compared with the control group, the AFC (**Figure 2A**) and OVD (**Figure 2B**) showed more significant increase (both P<0.001), and the ovarian stroma RI (**Figure 2C**) revealed more significant decrease after treatment in the test group (P<0.001).

We further analyzed the differences in the levels of E2 and progesterone in the mid-luteal phase between the two groups. Compared with the control group, the E2 and progesterone levels in the mid-luteal phase of the test group were considerably increased in the test group (both P<0.001). See **Table 2**.

The clinical symptoms and QOL were more significantly improved in the test group

Compared before treatment, the Kupperman indices of the two groups were significantly reduced after 3 and 6 months' treatment (all P<0.001). Compared with the control group, the Kupperman indices of the test group were significantly decreased after 3 and 6 months' treatment (P<0.01 or 0.001; **Figure 3**). Re-

Table 2. The levels of E2 and progesterone in the mid-luteal phase of the two groups of patients ($\bar{x}$ ± sd)

Group	Mid-luteal phase E2 (pmol/L)	Mid-luteal phase progesterone (nmol/L)
Test group (n=40)	170.64±22.79	43.18±9.05
Control group (n=40)	128.42±18.17	34.06±8.24
T score	9.161	4.713
P value	<0.001	<0.001

Note: E2: estradiol.

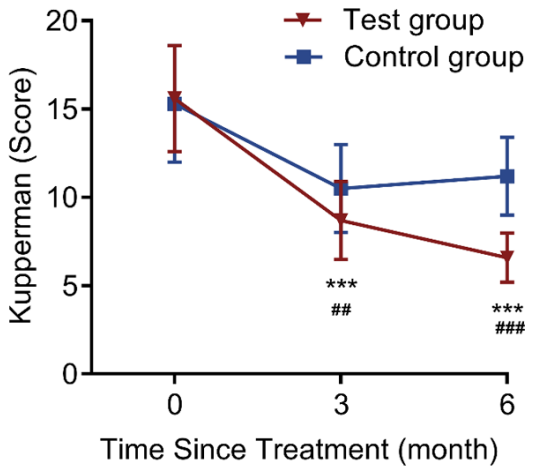

Figure 3. Kupperman scores before and after treatment in the two groups. Compared with before treatment, ***P<0.001; compared with the control group after treatment, ##P<0.01; ###P<0.001.

garding QOL, there were no significant differences in terms of MENQOL scores between the two groups before treatment (P>0.05). After treatment, MENQOL scores of the two groups were significantly lower than those of before treatment (all P<0.001). Compared with the control group, the MENQOL score of the test group was significantly decreased after treatment (P<0.001). In addition, the difference regarding MENQOL scores before and after treatment in the test group was significantly greater than that of the control group (21.85±4.64 vs. 10.36±5.03) (P<0.001). See **Table 3**.

Adverse effects and pregnancy rate within 1 year

During the treatment 2 cases of nausea and vomiting (5.00%) and 3 cases of breast pain (7.50%) were observed in the control group, which, however, were both recovered without additional treatment. No adverse effects were observed in the test group. There were no statistical differences regarding adverse effects between the two groups (P>0.05). Within 1 year of follow-up, 19 patients in the test group were successfully pregnant with a pregnancy rate of 47.50%; 10 patients in the control group were successfully pregnant with a rate of 25.00%. Compared with the control group, the pregnancy rate was significantly increased in the test group (P=0.036).

Discussion

Similar to the hormone sequential therapy, kidney-tonifying via TCM sequential therapy applies "Zhuyun Tang" from the 5th to 15th day of the menstrual cycle and "Guchong Tang" for the rest 12 days. Zhuyun Tang is composed of 12 TCM ingredients including Semen cuscutae, Fructus lycii, Angelica biserrata etc., which can effectively promote the growth of the endometrium and follicle development. Based on the characteristics of menstrual cycle, Guchong Tang modifies constituents on the basis of Zhuyun Tang by adding Fructus ligustri lucidi, Eclipta prostrata, and Rhizoma dioscoreae, which can effectively promote ovarian ovulation and maintain corpus luteum function. In this study, we have performed a prospective and randomized controlled research to investigate the effects and differences of sequential therapy for kidney-tonifying via TCM and sequential treatment of artificial menstrual cycle via the administration of estrogen and progesterone on the treatment of DOR and their impact on the QOL of patients.

Our results demonstrate that sequential therapy for kidney-tonifying via TCM could further improve sex hormone levels and ovarian reserve in women with DOR. Compared with the control group, serum FSH and LH levels are significantly reduced after treatment in the test group, while E2 and AMH levels are significantly upregulated. Previous studies have also shown that the treatment through kidney-tonifying TCM can effectively regulate the level of sex hormones in women with irregular menstruation and infertility [19, 20]. The ultrasound examination has revealed that AFC and OVD in the test group are more significantly increased

Table 3. The QOL of two groups of patients ($\bar{x}$ ± sd)

Group	Case	MENQOL		
		Before treatment	After treatment	Difference
Test group	40	72.45±13.61	50.15±10.35***	21.85±4.64
Control group	40	73.21±15.58	62.33±11.76***	10.36±5.03
T score		0.232	4.917	10.619
P value		0.817	<0.001	<0.001

Note: Compared before treatment with the group, ***P<0.001. MENQOL: Menopause-specific quality of life questionnaire; QOL: quality of life.

than those of the control group, which clearly reflects the significant improvement of ovarian function in patients with DOR by the sequential treatment for kidney-tonifying via TCM. We propose that compared with hormone therapy, the advantage of sequential therapy for kidney-tonifying via TCM is that this approach functions by recuperating the body of DOR patients inwardly. Different from the replacement effect of the hormone therapy, kidney-tonifying TCM regulates sex hormones by adjusting and improving the function of patients' own hypothalamic-pituitary-ovarian axis [21, 22]. At the same time, a study has reported that kidney-tonifying TCM can also upregulate the expression of hormone receptors, which may help improve the response of ovarian tissue [23]. In addition, a variety of blood-activating components in kidney-tonifying TCM can improve the pelvic blood microcirculation, which increases the blood supply to the ovaries and uterus and maintains their normal physiological functions [24, 25].

By comparing the Kupperman indices of the two groups after 3 and 6 months' treatment, we have demonstrated that the Kupperman index of the control group has not been improved but increased after the medication withdrawn, which has also been confirmed by other studies [26, 27]. In contrast, the Kupperman index has been improved in the test group 3 months after the medication withdrawn. This data suggests that sequential therapy for kidney-tonifying via TCM can effectively prevent the recurrence of this disease after hormone replacement therapy is ceased, but its specific mode of action still needs to be further investigated. Compared with the control group, the MENQOL score has also been significantly improved in the test group and no adverse effects are observed, which indicates that the sequential treatment for kidney-tonifying via TCM can effectively improve the clinical symptoms and QOL of DOR patients.

In clinical practice, most DOR patients not only seek help to improve their QOL, but many patients want to achieve or retrieve better reproductive function. E2 and progesterone levels in the mid-luteal phase are major evaluation indices of ovulation quality. Ganesh et al. have also elucidated that the upregulation of E2 levels in the middle and late stages of the corpus luteum indicates a higher pregnancy rate [28]. In this study, we have evaluated the E2 and progesterone levels in the mid-luteal phase and pregnancy rate within 1 year after treatment. Compared with the control group, the E2 and progesterone levels in the mid-luteal phase are significantly increased in the test group, suggesting a higher ovulation quality in the test group. Meanwhile, the pregnancy rate within 1 year in the test group has also been shown significantly higher than that in the control group (47.50% vs. 25.00%). This result further suggests that sequential treatment for kidney-tonifying via TCM can effectively improve the quality of ovulation and increase the pregnancy rate of DOR patients.

There are some limitations in this study. For example, the follow-up time is short, which does not cover the effect of the kidney-tonifying TCM treatment on patients' long-term outcome; this study does not further explore the specific mechanism of this sequential treatment strategy on patients' hormone induction and ovarian function. These aspects are worthy of further investigations. In addition, in terms of pregnancy rate, the fertility of males has not been evaluated, which may bias the results of the pregnancy rate of the two groups.

In summary, this study has demonstrated that the sequential therapy for kidney-tonifying via TCM can effectively improve the level of sex hormones and ovarian function, increase the fertility, and restore a higher pregnancy rate in patients with DOR, which, at the same time, could effectively improve the QOL of DOR patients in the long run. Sequential therapy for kidney-tonifying via TCM may provide an ideal treatment alternative for DOR patients.

Disclosure of conflict of interest

None.

Address correspondence to: Wenxu Duan, Department of Gynecology, Tianjin Nankai Hospital, No.6 Changjiang Road, Tianjin 300000, China. Tel: +86-15822741395; E-mail: duanwenxu02nk@163.com

References

[1] Xu Y, Nisenblat V, Lu C, Li R, Qiao J, Zhen XM and Wang SY. Pretreatment with coenzyme Q10 improves ovarian response and embryo quality in low-prognosis young women with decreased ovarian reserve: a randomized controlled trial. Reprod Biol Endocrinol 2018; 16: 29.

[2] Buigues A, Marchante M, Herraiz S and Pellicer A. Diminished ovarian reserve chemotherapy-induced mouse model: a tool for the preclinical assessment of new therapies for ovarian damage. Reprod Sci 2019; 7: 1609-1619.

[3] Huang K, Hu Y, Wu Y, Zhonh J, Wang S, Lin Z and Pan S. Hyperchloremia is associated with poorer outcome in critically ill stroke patients. Frontiers Neurol 2018; 9: 485.

[4] Amanvermez R and Tosun M. An update on ovarian aging and ovarian reserve tests. Int J Fertil Steril 2016; 9: 411-415.

[5] Du X, Xu L, Wang LJ, Heng ML, Bu HE, Hao Y and Tian JH. Comparison of the effect and safety of Kuntai capsule and hormone replacement therapy in patients with perimenopausal syndrome: a systematic review and Meta-analysis. J Tradit Chin Med 2017; 37: 279-285.

[6] Gleicher N and Barad DH. Dehydroepiandrosterone (DHEA) supplementation in diminished ovarian reserve (DOR). Reprod Biol Endocrinol 2011; 9: 67.

[7] Kuroda K, Ezoe K, Kato K, Yabuuchi A, Segawa T, Kobayashi T, Ochiai A, Katoh N and Takeda S. Infertility treatment strategy involving combined freeze-all embryos and single vitrified-warmed embryo transfer during hormonal replacement cycle for in vitro fertilization of women with hypogonadotropic hypogonadism. J Obstet Gynaecol Res 2018; 44: 922-928.

[8] Ried K. Chinese herbal medicine for female infertility: an updated meta-analysis. Complement Ther Med 2015; 23: 116-128.

[9] Qian LQ, Wang B, Niu JY, Gao S and Zhao DY. Assessment of the clinical effect of Chinese medicine therapy combined with psychological intervention for treatment of patients of perimenopausal syndrome complicated with hyperlipidemia. Chin J Integr Med 2010; 16: 124-130.

[10] Ke J and Duan R. Effects of flavonoids from semen cuscutae on the hippocampal-hypothalamic-pituitary-ovarian sex hormone receptors in female rats exposed to psychological stress. Clin Exp Obstet Gynecol 2013; 40: 271-274.

[11] Ahmad A, Tandon S, Xuan TD and Nooreen Z. A review on phytoconstituents and biological activities of cuscuta species. Biomed Pharmacother 2017; 92: 772-795.

[12] Zhang L, Ge Y, Li J and Chang YX. Simultaneous determination of columbianetin-β-d-glucopyranoside and columbianetin in a biological sample by high-performance liquid chromatography with fluorescence detection and identification of other columbianetin-β-d-glucopyranoside metabolites by ultra high - performance liquid chromatography coupled with quadrupole-time of flight mass spectrometry. J Pharm Biomed Anal 2018; 153: 221-231.

[13] Ma JH, Huang J, Hua SY, Zhang Y, Zhang YW, Li TT, Dong L, Gao AH and Fu XY. The ethnopharmacology, phytochemistry and pharmacology of angelica biserrata - a review. J Ethnopharmacol 2019; 231: 152-169.

[14] Zhang JH, Xin HL, Xu YM, Shen Y, He YQ, Hsien Y, Lin B, Song HT, Juan L, Yang HY, Qin LP, Zhang QY and Du J. Morinda officinalis how. - a comprehensive review of traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 2018; 213: 230-255.

[15] Lin J, Li XL, Song H, Li Q, Wang MY, Qiu XM, Li DJ and Wang L. A general description for Chinese medicine in treating premature ovarian failure. Chin J Integr Med 2017; 23: 91-97.

[16] 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.

[17] Davis SR. The kupperman index undressed. Maturitas 2019; 126: 90-91.

[18] Papadima EI, Boutsiadis A, Soldatou A, Lvanidou S, Vassilakou T and Michala L. Linguistic translation and validation of the menopause-specific quality of life (MENQOL) questionnaire in Greek menopausal women. Menopause 2020; 27: 808-815.

[19] Li HF, Shen QH, Chen WJ, Chen WM, Feng ZF and Yu LY. Efficacy of traditional Chinese medicine tonifying kidney (Bushen) and activating blood (Huoxue) prescription for premature ovarian insufficiency: a systematic review and meta-analysis. Evid Based Complement Altern Med 2020; 2020: 1789304.

[20] Liu W, Nguyen TN, Tran Thi TV and Zhou S. Kuntai capsule plus hormone therapy vs. hormone therapy alone in patients with premature ovarian failure: a systematic review and meta-analysis. Evid Based Complementary Altern Med 2019; 2019: 2085804.

[21] Keremu A, Yaoliwasi A, Tuerhong M, Kadeer N, Heyi, Yiming A and Yilike X. Research on the establishment of chronic stress-induced premature ovarian failure the rat model and effects of Chinese medicine Muniziqi treatment. Mol Reprod Dev 2019; 86: 175-186.

[22] Song KK, Ma WW, Huang C, Ding JH, Cui DD, Tan XJ, Xiao J and Zhang MM. Effect and mechanism of Bushen Huoxue recipe on ovarian reserve in mice with premature ovarian failure. J Huazhong Univ Sci Technol Med Sci 2016; 36: 571-575.

[23] Li LY, Lu SL, Ma QM, Wan PC and Liu CB. The comparison of reproductive hormone receptor expressions of the sheep ovary and hormone concentrations in two Chinese breeds. Reprod Domest Anim 2019; 54: 892-901.

[24] Huang C, Song K, Ma W, Ding J, Chen Z and Zhang M. Immunomodulatory mechanism of bushen huoxue recipe alleviates cyclophosphamide-induced diminished ovarian reserve in mouse model. J Ethnopharmacol 2017; 208: 44-56.

[25] Tang CL, Li F, Sun L and Li DJ. Therapeutic effect of Bushen Huoxue recipe on autoimmune premature ovarian failure mice established by immunization with recombinant porcine zona pellucida 4 antigen. Chin J Integr Med 2013; 19: 439-445.

[26] Burgos N, Cintron D, Latortue-Albino P, Serrano V, Rodriguez Gutierrez R, Faubion S, Spencer-Bonilla G, Erwin PJ and Murad MH. Estrogen-based hormone therapy in women with primary ovarian insufficiency: a systematic review. Endocrine 2018; 59: 235.

[27] Song Y, Xu W, Chatooah ND, Chen JF, Huang YZ, Chen PQ, Lan YB, Li CM, Ying Q, Ma LJ and Zhou JH. Comparison of low dose versus ultra-low dose hormone therapy in menopausal symptoms and quality of life in perimenopause women. Gynecol Endocrinol 2020; 36: 252-256.

[28] Ganesh A, Goswami S, Chattopadhyay R, Chakraborty C, Chaudhury K and Chakravarty BN. Luteal phase estradiol level: a potential predictive marker for successful pregnancy in in vitro fertilization/intracytoplasmic sperm injection. Fertil Steril 2009; 91: 1018-1022.