Effectiveness of nourishing Yin and tonifying Yang sequential therapy in combination with Climen on diminished ovarian reserve: a retrospective study

Sun Yuying, Tan Yong, Chen Shuping

**Abstract**

**OBJECTIVE:** To evaluate the clinical efficacy of nourishing Yin and tonifying Yang sequential therapy (NYTYST) in combination with the Western Medicine Climen in treating diminished ovarian reserve (DOR).

**METHODS:** From September 2014 to September 2016, 46 patients with DOR who received NYTYST and Climen (treatment group) and 51 patients who received Climen only (control group) at the Jiangsu Provincial Traditional Chinese Medicine Hospital (Nanjing, China) were retrospectively reviewed. Before and at 3 months after treatment, serum levels of follicle-stimulating hormone (FSH), FSH/LH, estradiol (E₂), and anti-Mullerian hormone (AMH), the antral follicle count (AFC), stromal peak systolic velocity (PSV), symptom scores, and the clinical effectiveness rate were measured and compared between the two groups.

**RESULTS:** After treatment, serum levels of FSH, FSH/LH and E₂, and symptom scores were significantly decreased compared with before treatment (all \( P < 0.05 \)) in the two groups. AMH levels, AFC, and PSV were significantly increased after treatment compared with before treatment in both groups (all \( P < 0.05 \)). Serum levels of FSH, FSH/LH, and E₂ after treatment were significantly lower in the treatment group than in the control group (all \( P < 0.05 \)). However, AMH levels and the AFC were significantly higher after treatment in the treatment group than in the control group (both \( P < 0.05 \)). The clinical effectiveness rate was significantly higher in the treatment group than in the control group (87% vs 68.6%, \( P < 0.05 \)).

**CONCLUSION:** NYTYST combined with Climen may be more effective in treating DOR than Climen alone.

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**Keywords:** Diminished Ovarian reserve; Nourishing Yin; Reinforcing Yang; Sequential therapy; Estradiol valerate, cyproterone acetate drug combination; Retrospective studies

**INTRODUCTION**

Ovarian reserve refers to the quantity and quality of recruitable follicles reserved in the ovary, and it reflects the female fertility potential and reproductive endocrine function. Diminished ovarian reserve (DOR) is
defined as a decrease in quantity and quality of the remaining ovarian follicular pool. Clinically, DOR mainly results in infertility, oligomenorrhea, light menstruation, and amenorrhea, which seriously threaten women’s reproductive health and quality of life. DOR leads to a limited remaining reproductive life span and a large amount of stress in patients. Moreover, a decline in ovarian hormones by DOR may cause other adverse events in women, such as increased bone turnover, low bone mineral density, disturbed sleep, and sexual dissatisfaction. If not treated in time, DOR may develop to premature ovarian failure. At present, routine treatment for DOR mainly includes hormone replacement treatment, dehydroepiandrosterone therapy, and growth hormone therapy. A 12-week treatment of Climen, which is a combination of estradiol valerate and cyproterone acetate, before in vitro fertilization increases the effect of dehydroepiandrosterone on DOR. This significantly enhances anti-Mullerian hormone (AMH) levels and decreases follicle-stimulating hormone (FSH) levels. However, these treatments are accompanied by clinical risks, such as endometrial cancer, breast cancer, and cardiovascular and cerebrovascular diseases. Because of the multiple bio-active components and few side effects, Traditional Chinese Medicine (TCM), such as electroacupuncture and TCM prescriptions, has been applied in treating DOR. TCM improves the ovarian reserve of patients. Huang et al. effectively treated DOR by nourishing kidney Yin at the follicular phase, nourishing Yin and Yang at the ovulatory phase, invigorating Yang at the luteal phase, and stimulating blood flow for improving menstruation cycle disorders. To determine better treatment efficacy, integrative medicine combining TCM prescriptions and Western Medicine (e.g., Climen) has been used, with satisfactory efficacy. Zhang et al. used the TCM ovulation Tiaojing Cuyun pill for 3 months after sequential artificial cycle therapy (estradiol valerate and progesterone) for infertile patients with DOR and achieved satisfactory outcomes. Zhang et al. combined estradiol valerate tablets, ethinyl, and TCM-based detoxification of the kidney. These authors found that this treatment promoted the blood circulation to improve ovarian function and increase the pregnancy rate of infertile patients with DOR. Nourishing Yin and tonifying Yang sequential therapy (NYTYST), which is based on the theory of TCM, is used to improve menstruation cycle disorders. NYTYST mobilizes and coordinates function of the human viscer, Qi, and blood, and recovers and establishes a normal menstruation cycle, thereby improving a woman’s reproductive function. NYTYST has been used for treating polycystic ovary syndrome and premature ovarian failure. However, the efficacy of NYTYST in DOR has not been evaluated yet. Therefore, in this study, we investigated the effect of NYTYST in combination with Climen for treating DOR.

MATERIALS AND METHODS

Patients
Between September 2014 and September 2016, 130 patients with DOR who were treated using NYTYST and Climen or only using Climen at the Department of Reproductive Medicine, Jiangsu Provincial Hospital of Traditional Chinese Medicine, were primarily retrospectively reviewed. We included the following infertile patients: (a) those who were diagnosed with DOR and with the requirement of fertility; (b) those aged 20-40 years; (c) those who were not administrated hormonal drugs within 3 months before treatment, and (d) those with complete clinical information. Exclusion criteria were as follows: (a) patients with endocrine diseases, such as polycystic ovarian syndrome, hyperprolactinemia, hyperandrogenism, hypothyroidism, and adrenal dysfunction, which affect ovulation; (b) patients with endometriosis or adenomyosis; (c) patients with DOR due to iatrogenic factors, such as pelvic surgery, radio-/chemotherapy for malignant tumors and uterine arterial embolization therapy; (d) patients combined with severe primary medical, surgical, mental, or other diseases; (e) patients who are sensitive to components of the prescriptions used in this study; and (f) patients with infertility attributable to galactosemia, mucopolysaccharide diseases, or other genetic factors. Selection of the treatment regimen was according to the patients’ wishes. For patients who had good compliance with and clear willingness to adhere to TCM therapy and who could afford the corresponding medical expenses, NYTYST in combination with Climen was orally taken. In contrast, other patients who were not willing to take TCM prescriptions for various reasons were treated with routine Climen alone. A total of 46 eligible patients who received NYTYST and Climen (treatment group) and 51 who only received Climen (control group) were studied. This study was approved by the Ethics Committee of Jiangsu Provincial Hospital of Traditional Chinese Medicine. Written informed consent was obtained from each patient.

Diagnosis
There is no consensus on the criteria for diagnosis of DOR. Therefore, DOR was determined according to the 2015 Committee Opinion of the American College of Obstetricians and Gynecologists and 2011 European Society of Human Reproduction and Embryology consensus on the definition of a "poor response" to ovarian stimulation for in vitro fertilization. In brief, DOR was determined according to the clinical manifestation (including infertility, menstruation disorder, and perimenopausal syndrome) and at least two of the five following biochemical characteristics: (a) AMH levels < 1.1 ng/mL, (b) basic antral follicle count (AFC) < 5, (c) b follicle stimulating hormone (FSH) levels > 10 and < 40 IU/L, (d) basic estradiol (E2) lev-
Serum FSH, LH, and E2 levels, the AFC, and stromal peak systolic velocity (PSV) are widely used to assess ovarian function. Nourishing Yin and tonifying Yang prescriptions were orally administrated twice daily from the fifth day of the menstruation cycle to the ovulatory period. Before the menstruation cycle, tonifying Yang prescriptions were made up with water and then orally taken twice daily. This prescription was then administered one pill daily from the fifth day of the menstruation cycle or withdrawal of bleeding, continuously for 21 d every month, for a total of 3 months. Patients in the treatment group also had regular administration of Climen as in the control group. Furthermore, patients in the treatment group had a nourishing Yin prescription, including 10 g Dihuang (Radix Rehmanniae), 10 g Shanzhuye (Fructus Macrocarpi), 10 g Guijia (Canapax et Plastrum Testudinis), 10 g Tusizi (Semen Cucurbitae), 10 g Ziheche (Placenta Hominis), 10 g Danggui (Radix Angelicae Sinensis), and 10 g Baishao (Radix Paeoniae Alba). This prescription was made up with water and then orally taken twice daily from the fifth day of the menstruation cycle to the ovulatory period. Before the menstruation cycle, tonifying Yang prescription, including 10 g Baijitian (Radix Morindae Officinalis), 10 g Chuanxuduan (Radix Dioscoreae), 10 g Buguzhi (Fructus Poriae), 10 g Yinyanghuo (Herba Epimedi Brevicornus), 10 g Dangshen (Radix Codonopis), and 10 g Shanyao (Rhizoma Dioscoreae Opposita), was made up with water and orally administered twice daily from the 16th day of menstruation cycle to the start of the next menstruation. Nourishing Yin and tonifying Yang prescriptions were taken by patients continuously for 3 months.

Assessment

Symptoms of DOR were evaluated by the Scale of Symptoms of Kidney Deficiency, which has been widely applied in TCM (16, 17). Serum FSH, AMH, FSH/LH, and E2 levels, the AFC, and stromal peak systolic velocity (PSV) are widely used to assess ovarian reserve. Serum FSH, LH, and E2 levels were measured by the chemiluminescence microparticle immuno assay method using the corresponding kit (Abbott Diagnostics, Ireland), and AMH levels were measured by an electrochemiluminescence assay (Roche Diagnostics GmbH, Mannheim, Germany). The AFC and PSV were observed under a vaginal ultrasound machine (IU22; Philips, Bothell, WA, USA). The effect of treatment was assessed according to the criteria as described previously in the Guideline of New Drug Clinical Research of Traditional Chinese Medicine. A clinical cure was defined as follows: (a) the period, color, amount, and quality of menstruation returned to normal, accompanying symptoms disappeared, and serum levels of FSH, LH, and E2 were within the normal range, AMH levels were ≥ 1.1 ng/mL and the AFC increased to 8-12; and (b) infertile patients became pregnant during or within 1 year after the 3-month treatment. Clinical effectiveness was defined as follows: menstruation appeared intermittently and accompanying symptoms improved, serum levels of FSH, LH, E2, and AMH were close to the normal range, and the AFC increased to 5-6. Ineffectiveness was determined if no significant improvement of clinical symptoms or other biochemical parameters were observed. The efficient rate was determined as the proportion of patients who obtained a cure or effectiveness among the total patients with DOR.

**RESULTS**

### Clinical characteristics of the patients

Ninety-seven patients with DOR were eventually analyzed. There were 46 patients in the treatment group and 51 in the control group. The patients’ mean age was (29.8 ± 3.4) years (range, 19-39 years) in the treatment group, with a mean disease duration of (2.8 ± 0.9) years (range, 1-4 years). The patients’ mean age was (30.2 ± 3.4) years (range, 20-28 years) in the control group, with a mean disease duration of (3.1 ± 1.1) years (range, 1-5 years). There were no significant differences in age, duration of disease, infertility, oligomenorrhea, and secondary amenorrhea between the treatment and control groups (all P > 0.05, Table 1).

### Changes in blood hormone levels

There were no significant differences in the baseline blood levels of FSH, FSH/LH, E2, and AMH between the treatment and control groups (Table 2). In both groups, blood levels of FSH, FSH/LH, and E2 after treatment were significantly decreased compared with those before treatment, while AMH levels were signifi-

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**TCM classification of DOR**

As described previously, kidney Yin deficiency was determined as follows: (a) main symptoms included primary or secondary infertility, an advanced or delayed menstruation cycle, hypomenorrhea, red and thick menstruation, and soreness and weakness of the waist and knees; (b) minor symptoms included hectic fever and sweating, insomnia and dreaminess, being up- and restless, physically and mentally fatigued, irritable, dizziness and tinnitus, dark or light red tongue, thin and white tongue fur, and with a thin or obscure pulse.
cantly increased (all \( P < 0.05 \), Table 2). Additionally, after treatment, blood levels of FSH, FSH/LH, and E2 in the treatment group were significantly lower than those in the control group, while AMH was significantly higher (all \( P < 0.05 \), Table 2). Moreover, the decrease in blood levels of FSH, FSH/LH, and E2, as well as the increase in AMH levels after treatment compared with before treatment were significantly greater in the treatment group than in the control group (all \( P < 0.05 \), Table 2).

**Changes in the symptom scores, AFC, and stromal PSV**

There were no significant differences in the baseline symptom scores, AFC, and stromal PSV between the treatment and control groups. After treatment, the symptom scores in both of the groups were significantly decreased, while the AFC and PSV were significantly increased compared with before treatment (all \( P < 0.05 \), Table 3). The AFC after treatment in the treatment group was significantly higher than that in the control group (\( P < 0.05 \), Table 3). Furthermore, the increase in the AFC after treatment compared with before treatment was significantly greater in the treatment group than in the control group (\( P < 0.05 \)).

### Clinical evaluation

The treatment group had a total efficient rate of 87% (40/46 patients), which was significantly higher than that in the control group (68.6%, 35/51 patients) (\( \chi^2 = 4.63, P < 0.05 \), Table 4).

### DISCUSSION

With regard to TCM theory, the kidney stores essence, cultivate renal Yin and renal Yang, and dominates reproduction. Ebb and flows of the kidney’s vital essence affect waxing and waning of the menstruation cycle and the resultant fertility capacity. Therefore, the kidney is responsible for initiation, development, and decline of the female reproductive axis, and determines ovarian physiological function and female reproductive health. Ding et al.\(^2\) suggested that kidney deficiency is the main pathological mechanism of DOR, and clinically, DOR can be treated by using the tonifying kidney method combined with impact therapy and toning the body. Li et al.\(^2\) used the nourishing kidney Yin method to effectively improve the status of DOR. According to kidney Yin and Yang transformation, rhythm of growth and decline, and changes in waxing and waning of Qi-blood at different phases of the men-

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**Table 1 Clinical characteristics of the treatment and control groups (x ± s)**

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment group (( n = 46 ))</th>
<th>Control group (( n = 51 ))</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.8±3.4 (range, 19-39)</td>
<td>30.2±3.4 (range, 20-38)</td>
<td>0.897</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>2.8±0.9 (range, 1-4)</td>
<td>3.1±1.1 (range, 1-5)</td>
<td>0.184</td>
</tr>
<tr>
<td>Primary infertility [n (%)]</td>
<td>20 (43.5)</td>
<td>23 (45.1)</td>
<td>0.873</td>
</tr>
<tr>
<td>Secondary infertility [n (%)]</td>
<td>26 (56.5)</td>
<td>28 (54.8)</td>
<td>0.873</td>
</tr>
<tr>
<td>Oligomenorrhea [n (%)]</td>
<td>31 (67.4)</td>
<td>40 (78.4)</td>
<td>0.22</td>
</tr>
<tr>
<td>Secondary amenorrhea [n (%)]</td>
<td>15 (32.6)</td>
<td>11 (21.6)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Notes: control group: patients received Climen one pill daily from the fifth day of the menstruation cycle or withdrawal of bleeding, continuously for 21 d every month, for a total of 3 months. Treatment group: patients had regular administration of Climen as in the control group and had nourishing Yin prescription one dose daily from the fifth day of the menstruation cycle to the ovulatory period and tonifying Yang prescription one dose daily from the 16th day of menstruation cycle to the start of the next menstruation, continuously for 3 months. FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: estradiol; AMH: anti-Mullerian hormone. *\( P < 0.05 \) compared with baseline data of the control group; †\( P < 0.05 \) compared with baseline data of the treatment group; ‡\( P < 0.05 \) compared with post-treatment data of the control group; §\( P < 0.05 \) compared with changes in the control group.
which kidney is the material base and kidney Yang oocytes are considered as kidney-stored essence, for growth, and thicken intimal endometrium. In TCM, Yin placenta is also included in these prescriptions. All of peony root enrich the blood sea. Additionally, human-rich essence and nourish the kidney. dodder nour annia root, dogwood, and processed tortoise shell en.

Among post-menstruation prescriptions, dried Rehm

uration of eggs, which are the basis for ovulation.

during the interval of the menstruation cycle, Yin grows extensively, which in turn transforms into Yang. During this period, in the condition of Yin growth and development and maturation of oocytes, Yin and Yang transform into each other. As a result, the blood-gas changes violently to retrieve oocytes. Yin essence is vigorous, and it transforms essence into Qi, as well as Yin into Yang. The ovary then produces corpus luteum and secretes progesterone when Yang grows and is dominant. The premenstruation period features growth of Yang and a decline in Yin. Growth of Yang not only provides warm nourishment and transfer of Yang for secreted eggs during the ovulation period to prepare for pregnancy, but also promotes growth and development of reproductive function. The premenstruation period, especially in the first half, is equivalent to the implantation window time of fertilized eggs in Western Medicine, and this time is critical for outcome of the period. Therefore, we should nourish Yang and warm uterine to assist in pregnancy or normalization of menstruation. In premenstruation prescriptions, Morinda officinalis, psoralen, and Epimedium tonify kidney Yang. Ruc Kommia and teasel tonify the liver and kidney, and Codonopsis pilosula regulates Qi-blood. Chinese yam promotes Yang transformation from Yin to prevent damage of Yin by warming. All of these components coordinately tonify the kidney and Yang, and improve luteal function to provide a good internal environment for implantation of fertilized eggs.

Sequential therapy using pre- and postmenstruation prescriptions are used in treating DOR, polycystic ovary syndrome, and other reproductive endocrine diseases through reestablishment of the menstruation cycle. Shen et al.31 showed that NYTYST combined with in vitro fertilization-embryo transplantation improved sexual gland function, increased the quality of oocytes and embryos, and reduced adverse reactions in treatment of infertility. Wang et al.32 showed that NYTYST reduced serum sexual hormone levels (testosterone, E2), and se-

Table 3 Changes in symptom scores, AFC, and PSV in the treatment and control groups (x ± s)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control group (n = 51)</th>
<th>Treatment group (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before the treatment</td>
<td>After the treatment</td>
</tr>
<tr>
<td>Symptom scores</td>
<td>11.8±1.6</td>
<td>3.6±1.6</td>
</tr>
<tr>
<td>AFC</td>
<td>2.4±0.7</td>
<td>5.8±1.2</td>
</tr>
<tr>
<td>PSV</td>
<td>9.6±0.7</td>
<td>12.5±0.7</td>
</tr>
</tbody>
</table>

Notes: control group: patients received Climen one pill daily from the fifth day of the menstruation cycle or withdrawal of bleeding, continuously for 21 d every month, for a total of 3 months. Treatment group: patients had regular administration of Climen as in the control group and had nourishing Yin prescription one dose daily from the fifth day of the menstruation cycle to the ovulatory period and tonifying Yang prescription one dose daily from the 16th day of menstruation cycle to the start of the next menstruation, continuously for 3 months. AFC: antral follicle count; PSV: peak systolic velocity. \( P < 0.05 \) compared with baseline data of the control group; \( P < 0.05 \) compared with baseline data of the treatment group; \( P < 0.05 \) compared with changes in the control group.

Table 4 Clinical evaluation of the treatment and control groups [n (%)]

<table>
<thead>
<tr>
<th>Item</th>
<th>Control group (n = 51)</th>
<th>Treatment group (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before the treatment</td>
<td>After the treatment</td>
</tr>
<tr>
<td>Cure</td>
<td>6 (11.8)</td>
<td>7 (15.2)</td>
</tr>
<tr>
<td>Effectiveness</td>
<td>29 (56.8)</td>
<td>33 (71.8)</td>
</tr>
<tr>
<td>Efficient rate</td>
<td>35 (68.6)</td>
<td>40 (87%)</td>
</tr>
<tr>
<td>Inefficient</td>
<td>16 (31.4)</td>
<td>6 (13)</td>
</tr>
</tbody>
</table>

Notes: control group: patients received Climen one pill daily from the fifth day of the menstruation cycle or withdrawal of bleeding, continuously for 21 d every month, for a total of 3 months. Treatment group: patients had regular administration of Climen as in the control group and had nourishing Yin prescription one dose daily from the fifth day of the menstruation cycle to the ovulatory period and tonifying Yang prescription one dose daily from the 16th day of menstruation cycle to the start of the next menstruation, continuously for 3 months. AFC: antral follicle count; PSV: peak systolic velocity. \( P < 0.05 \) compared with baseline data of the control group; \( P < 0.05 \) compared with baseline data of the treatment group; \( P < 0.05 \) compared with changes in the control group.

After the menstruation cycle, women feel emptiness of Xuehai (blood sea) and deficiency of Yin essence, with the main physiological features of growth of Yin and a decline in Yang. Growth of Yin promotes development of eggs and sperm. Growth of Yin and sufficient water promote sperm and oocyte maturation, conservation of the blood sea, and growth of the endometrium. The main related treatment at this stage is nourishing Yin, focusing on helping growth, and development and maturation of eggs, which are the basis for ovulation. Among post-menstruation prescriptions, dried Rehmannia root, dogwood, and processed tortoise shell enrich essence and nourish the kidney. Yang dodder nourishes the kidney, neutralizes the kidney essence, and enhances the effect of filling essence. Angelica and white peony root enrich the blood sea. Additionally, human placenta is also included in these prescriptions. All of these components nourish Yin and blood, invigorate the kidney and enrich essence, promote follicular growth, and thicken intimal endometrium. In TCM, oocytes are considered as kidney-stored Yin essence, for which kidney Yin is the material base and kidney Yang promotes growth. Therefore, the main treatment at this stage is mainly to invigorate kidney Yin, nourish blood and Yin, and thus tonify essence (egg).
rum insulin and insulin-like growth factor levels in rats with polycystic ovary syndrome, which indicated a good treatment outcome. However, to date the effect of NYTYST prescriptions or their combination with Western Medicine in treating DOR has not been formally reported in international peer-reviewed journals. In this study, we showed that TCM-based NYTYST prescriptions in combination with the Western Medicine Cilmen significantly decreased serum levels of FSH, FSH/LH, and E2, and symptom scores, and increased AMH levels, AFC, and PSV in patients with DOR. Furthermore, this combination achieved a significantly higher clinical effectiveness rate compared with Cilmen alone. These findings suggest that NYTYST combined with Cilmen may be effective in treating DOR.

A limitation of this study is that it was a retrospective study in a single center, which might have resulted in bias of patient selection and incomplete information of some important clinical outcomes. Future prospective, multicenter, randomized, controlled studies are warranted to validate our results.

In conclusion, TCM-based NYTYST prescriptions in combination with Cilmen may be more effective in treating DOR than Cilmen alone. This combination improves ovarian reserve and might prevent development into premature ovarian failure.

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