Clinical Effects of Bushen Culuan Prescription on Infertility Due to Decreased Ovarian Reserve of Kidney Deficiency and Liver Depression Pattern

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Abstract

Objective The objective of this study was to explore the clinical effects of Bushen Culuan prescription on infertility due to decreased ovarian reserve of kidney deficiency and liver depression pattern.

Methods Totally 100 infertile patients with ovarian hypofunction treated from October 2019 to January 2022 were selected and divided into the observation group and the control group by a random number table. The control group was treated with estrogen sequential therapy, and the observation group was treated with Bushen Culuan prescription. The clinical efficacy, sex hormone levels including (follicle-stimulating hormone [FSH], luteinizing hormone [LH], and estradiol [E2]), ovarian function, ovarian blood flow status (peak systolic velocity [PSV], antral follicle count [AFC], arterial pulse index [PI], and resistance index [RI]), and pregnancy outcome in the two groups were compared and analyzed.

Results The total effective rate of the observation group (96.00%) was significantly higher than that of the control group (80.00%). The difference was statistically significant (p < 0.05). Before treatment, there was no significant difference in sex hormone levels (FSH, LH, E2, and FSH/LH), ovarian function (number of primary follicles, number of dominant follicles, ovulation number, and endometrial thickness), and ovarian blood flow (PSV, RI, PI, and AFC) between the two groups (p > 0.05). After treatment, the levels of serum hormones FSH, LH, E2, and FSH/LH decreased significantly in both groups. The number of primary follicles, dominant follicles, and ovulation increased significantly, and the thickness of endometrium decreased significantly; the indexes of PSV, RI, and AFC of ovarian blood flow increased significantly, and the PI index decreased significantly. The levels of each index in the observation group were better than those in the control group (p < 0.05). In the control group, 32 cases were pregnant within 1 year after treatment, and the pregnancy rate was 64.00%; 21 cases in the observation group were pregnant within 1 year after treatment, and the pregnancy rate was 42.00%; the pregnancy rate in the observation group was higher.
Introduction

The ovarian function reflects the ability of follicles in the ovarian cortex of the female reproductive and endocrine system to form high-quality oocytes, mainly referring to the number and quality of follicles in the ovary. Decreased ovarian reserve (DOR) refers to the decreased ability of the ovaries to produce high-quality oocytes, resulting in a decrease in female reproductive capacity, accompanied by pathological conditions such as abnormal hormone expression levels. In recent years, with the accelerated pace of life in modern society, the clinical incidence of DOR has increased year by year, bringing greater psychological pressure to the population of childbearing age. Therefore, urgent attention should be paid to reproductive capacity and the improvement of pregnancy status in modern society.

Modern medical research studies show that the pathogenesis of DOR is not clear. Clinically, it is believed that the imbalance of brain–heart (kidney)–uterus axis leads to the reduction of hormone regulation ability, and the inability to dynamically adjust the body's hormone level can lead to this disease. The clinical treatment is mainly hormone adjuvant therapy (such as dehydroepiandrosterone, testosterone gel, etc.), which uses the periodic changes of body hormones to regulate hormone secretion. Long-term use may reduce the self-adjustment ability of hormone, and the effect will not be good. The treatment of DOR in Chinese medicine is mainly characterized by syndrome differentiation, combined with the patient's signs and menstrual characteristics to prescribe medicine, and the clinical effect is obvious. Based on this, the treatment of infertility patients with DOR by Bushen Culuan prescription is reported as follows.

Data and Methods

General Data

Totally, 100 patients with infertility due to DOR admitted to the First Affiliated Hospital of Henan University of Chinese Medicine from October 2019 to January 2022 were selected and equally divided into the observation group and the control group using the random number table method. The age of the observation group was 36.5 ± 8.5 years, and the course of disease was (2.1 ± 1.3) years, with 30 cases of primary infertility and 20 cases of secondary infertility. The age of the control group was 37.5 ± 9.5 years old, and the course of disease was 3.2 ± 1.1 years, with 26 cases of primary infertility and 24 cases of secondary infertility. There was no significant difference in the general data between the two groups (p > 0.05), which was comparable.

Inclusion Criteria of Cases

In Western medicine, it meets the diagnostic criteria of ovarian reserve dysfunction, that is, 40IU·L⁻¹ follicle-stimulating hormone (FSH) > 10IU·L⁻¹ (menstrual cycle: 2–3 days), and the age from 35 to 40 years old; antral follicle count (AFC) < 5; and FSH/luteinizing hormone (LH) > 3.6. In Chinese medicine, it meets the diagnostic criteria of “infertility” and “syndrome of kidney deficiency and liver depression” in the Clinical Guidelines for New Chinese Medicine, that is, the main symptoms include irregular scanty menstruation or amenorrhea, dark red menstruation with clots, infertility. Secondary symptoms include lumbosacral aching, dizziness and tinnitus, decreased libido, abdominal distension or hypochondriac pain, breast distension and pain, emotional disturbance, irritability, frequent sighing, red tongue with white tongue coating, thin and sunken pulse, or sunken and wiry pulse. It is supposed to meet at least one main symptom and two or more secondary symptoms; tongue and pulse diagnoses were used as a reference. All patients included in the study were approved by the ethics committee of the hospital, and the patients or their families were informed and agreed to sign the informed consent form.

Exclusion Criteria of Cases

Excluded criteria were infertility due to organic ovarian lesions, complicated with severe cardiovascular and cerebrovascular diseases; patients having received hormone treatment in the past 3 months; and patients allergic to the drug used in the research or unsuitable for this treatment plan.

Therapies

Psychological counseling was given to the two groups, and the causes, development, and treatment of the disease were explained in details so as to remove the psychological interference between the husband and wife as far as possible, reduce their psychological pressure, enhance the confidence of patients and their families in the treatment, maintain a good psychological state and appropriate exercise, help in adjustment to living habits, and promote active cooperation with the treatment.

The control group was treated with estrogen sequential therapy. On the 5th day of menstruation, estradiol valproate...
Clinical Effects of Bushen Culuan Prescription on Infertility
Feng and Ma

Follicle Development and Endometrial Thickness
The follicular development and endometrial thickness (the detection time can be adjusted in due time according to the follicular development of patients) on the 5th day of the menstrual cycle before and after the end of the third cycle of treatment were measured by Doppler ultrasonography.13

Examination of Ovarian Blood Flow Indicators
Ovarian blood flow indicators, including peak systolic velocity (PSV), AFC, arterial pulse index (PI), and resistance index (RI) were examined by Doppler ultrasound.

Pregnancy Rate
The pregnancy within 1 year after treatment was statistically analyzed and the pregnancy results were compared between the two groups.

Statistical Methods
SPSS18.0 was used for statistical analysis. The measurement data were expressed in the form of (x ± s), and the normality and homogeneity of variance of the measurement data were checked. The data subjected to normal distribution were compared within and between groups using t-test. Nonparametric test was used for data not conforming to normal distribution. Count data were expressed in the form of rate (%), and χ² test was used. For all intergroup comparisons, p < 0.05 represented that the difference was statistically significant.

Results
Comparison of Clinical Efficacy Between Two Groups
The effective rate in the observation group (96.00%) was significantly higher than that in the control group (80.00%), and the difference was statistically significant (p < 0.05). The results are presented in Table 1.

Comparison of Serum Hormone Levels between the Two Groups
Before treatment, there was no significant difference in serum FSH, LH, E₂, and FSH/LH between the two groups (p > 0.05). After treatment, the levels of serum hormones FSH, LH, and FSH/LH in the two groups significantly decreased, and the levels of the indicators in the observation groups obviously decreased.

Table 1 Comparison of clinical efficacy between the two groups (n = 50, cases [%])

<table>
<thead>
<tr>
<th>Groups</th>
<th>Obviously effective</th>
<th>Effective</th>
<th>Ineffective</th>
<th>Total effective rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>27 (54.0)</td>
<td>21 (42.0)</td>
<td>2 (4.0)</td>
<td>48 (96.0)</td>
</tr>
<tr>
<td>Control group</td>
<td>16 (32.0)</td>
<td>24 (48.0)</td>
<td>10 (20.0)</td>
<td>40 (80.0)</td>
</tr>
<tr>
<td>χ²</td>
<td>4.640</td>
<td></td>
<td></td>
<td>0.031</td>
</tr>
</tbody>
</table>

Observation Indicators
Clinical Efficacy
The clinical efficacy was judged according to the criteria of the Clinical Guidelines for New Chinese Medicine. Significantly effectiveness: the clinical symptoms of the patients basically disappeared, and the serum FSH (5–40 mIU·mL⁻¹), E₂ (25–50 pmol·L⁻¹), LH (5–25 mIU·mL⁻¹) basically returned to normal levels; effectiveness: the clinical symptoms of the patients got improved, and the levels of serum FSH, E₂, and LH basically returned to normal, but there were still some abnormal indicators. Ineffectiveness: there were no significant changes in clinical symptoms and levels of serum FSH, E₂, and LH.

Effectiveness rate = (significantly effectiveness + effectiveness)/n × 100%

Comparison of Hormone Levels
The sex hormone levels of the patients before and after treatment in the third cycle were measured. The fasting venous blood of patients in the early morning was extracted, and the expression levels of FSH, LH, and E₂ were determined by electrochemical luminescence.

Comparison of hormone levels in the two groups (t < 0.05). After treatment, the levels of serum hormones FSH, LH, and FSH/LH in the two groups significantly decreased, and the levels of the indicators in the observation groups obviously decreased.

Table 1 Comparison of clinical efficacy between the two groups (n = 50, cases [%])

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<td></td>
<td>0.031</td>
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Follicles (1 mg) were taken orally once daily for 21 consecutive days. On the 15th day of menstruation, progesterone tablets (100 mg) were taken twice daily for 10 consecutive days. On the 5th day of withdrawal bleeding, they were readministered according to the above procedures for three consecutive menstrual cycles.

The observation group was treated with Bushen Culuan prescription, with herbs of Huangqi (Astragali Radix) 20 g, Shudihuang (Rehmanniae Radix Praeparata) 20 g, Tusizi (Cuscutae Semen) 15 g, Yinyanghuo (Epidemii Folioum) 15 g, Nyu Zhenzi (Ligustri Lucidi Fructus) 15 g, Mohanlian (Ecliptae Herba) 15 g, Danggui (Angelicae Sinensis Radix) 9 g, Chuanxiong (Chuanxiong Rhizoma) 6 g, Baishao (Paoniae Alba Radix) 6 g, processed Xiangfu (Cyperi Rhizoma Praeparata) 9 g, and processed Gancao (Glycyrrhizae Radix et Rhizoma Praeparata cum Melle) 6 g. The composition of the prescription is adjusted according to the changes in the menstrual period of the patients. In the early menstrual period, it mainly focuses on warming kidney yang by adding Bajitian (Morindae Officinalis Radix) and Roucongrong (Cistanches Herba); during menstruation, it mainly focuses on warming and dredging with Chishao (Paoniae Radix Rubra) and Danshen (Salviae Miltiorrhizae Radix et Rhizoma); at the later stage of menstruation, it mainly focuses on tonifying the kidney and nourishing blood by adding Gouqizi (Lycii Fructus) and Nyu Zhenzi (Ligustri Lucidi Fructus); in the ovulation period, it mainly focuses on tonifying the kidney and promoting blood circulation by adding Zelan (Lycopii Herba). The above herbs were soaked in water for 30 minutes and decocted twice, concentrated to approximately 500 mL after combining the decoction, and taken in the morning and evening, respectively, for three consecutive menstrual cycles.12
Table 2 Comparison of hormone levels before and after treatment between the two groups (n = 50, x ± s)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>FSH (mIU·mL⁻¹)</th>
<th>LH (mIU·mL⁻¹)</th>
<th>E₂ (pmol·L⁻¹)</th>
<th>FSH/LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>Before treatment</td>
<td>32.1 ± 9.5</td>
<td>24.2 ± 9.4</td>
<td>38.1 ± 9.8</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>18.8 ± 2.4</td>
<td>14.2 ± 3.6</td>
<td>87.2 ± 11.3</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Control group</td>
<td>Before treatment</td>
<td>33.4 ± 9.8</td>
<td>24.9 ± 9.3</td>
<td>42.2 ± 8.3</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>28.6 ± 3.1</td>
<td>17.3 ± 3.5</td>
<td>65.6 ± 12.5</td>
<td>1.7 ± 0.4</td>
</tr>
</tbody>
</table>

Abbreviations: E₂, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Comparison of Ovarian Blood Flow Before and After Treatment Between the Two Groups

Before treatment, there was no significant difference in PSV, RI, PI, and AFC indicators of ovarian blood flow between the two groups (p > 0.05). After treatment, the indicators of PSV, RI, and AFC of ovarian blood flow in the two groups significantly increased, and the PI indicators significantly decreased. The improvement of ovarian blood flow in the observation group was significantly better than that in the control group, and the difference between the two groups was statistically significant (p < 0.05). The results are presented in Table 4.

Table 3 Comparison of follicular development and endometrial thickness after treatment between the two groups (n = 50, x ± s)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>Number of primary follicles</th>
<th>Number of dominant follicles</th>
<th>Number of ovulations</th>
<th>Endometrial thickness/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>Before treatment</td>
<td>1.6 ± 0.6</td>
<td>0.7 ± 0.4</td>
<td>0.4 ± 0.2</td>
<td>6.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>3.8 ± 1.4</td>
<td>2.6 ± 0.8</td>
<td>1.5 ± 0.3</td>
<td>9.6 ± 1.5</td>
</tr>
<tr>
<td>Control group</td>
<td>Before treatment</td>
<td>1.7 ± 0.8</td>
<td>0.6 ± 0.3</td>
<td>0.3 ± 0.2</td>
<td>6.3 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>2.6 ± 1.1</td>
<td>1.1 ± 0.7</td>
<td>0.8 ± 0.2</td>
<td>8.2 ± 1.2</td>
</tr>
</tbody>
</table>

Note: The number of primary follicles were detected on day 5 of the menstrual cycle, with a diameter of approximately 7 mm; dominant follicles were those whose diameter were above 16 mm.

Comparison of Pregnancy Outcomes Between the Two Groups

Totally, 32 patients in the observation group got pregnant within 1 year after treatment, and the pregnancy rate was 64.00%. In the control group, 21 patients were pregnant within 1 year after treatment, and the pregnancy rate was 42.00%. The pregnancy rate in the observation group was statistically significantly higher than that in the control group, and the difference between the two groups was statistically significant (p < 0.05). The results are presented in Table 4.

Table 4 Comparison of ovarian blood flow before and after treatment between two groups (n = 50, x ± s)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>PSV/(cm/s)</th>
<th>PI/%</th>
<th>RI/%</th>
<th>AFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation group</td>
<td>Before treatment</td>
<td>9.6 ± 1.6</td>
<td>0.9 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>3.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>16.8 ± 1.7</td>
<td>0.6 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>9.2 ± 1.4</td>
</tr>
<tr>
<td>Control group</td>
<td>Before treatment</td>
<td>9.4 ± 1.4</td>
<td>0.9 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>3.4 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>After treatment</td>
<td>12.6 ± 1.8</td>
<td>0.8 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>6.1 ± 1.5</td>
</tr>
</tbody>
</table>

Abbreviations: AFC, antral follicle count; PI, arterial pulse index; PSV, peak systolic velocity; RI, resistance index.

Group were lower than those in the control group, with statistically significant differences (p < 0.05). The levels of E₂ in two groups significantly increased, and the levels of the indicators in the observation group were higher than those in the control group, with statistically significant differences (p < 0.05). The results are presented in Table 2.

Comparison of Follicular Development and Endometrial Thickness Between the Two Groups

Before treatment, there was no significant difference in the number of primary follicles, dominant follicles, and ovulation and endometrial thickness between the two groups (p > 0.05). After treatment, the number of primary follicles, dominant follicles, and ovulation in the two groups significantly increased, and the endometrial thickness significantly increased. The improvement of follicular development and endometrium in the observation group was better than that in the control group, and the difference between the two groups was statistically significant (p < 0.05). The results are presented in Table 3.
higher than that in the control group, and the difference was statistically significant ($\chi^2 = 4.014, p = 0.045 < 0.05$).

**Discussion**

In Chinese medicine, it is believed that the main pathogenesis of DOR involves kidney deficiency affecting multiple organs, and its clinical manifestations are mainly menstrual disorders with long-term infertility, which should be classified as categories of “late menstruation,” “amenorrhea,” “infertility.”\(^\text{14,15}\) The ancients said, “When kidney qi becomes prosperous, Tiangui begins to appear, and conception vessel and thoroughfare vessel are vigorous in function, and thus the female begin to have menstruation. Vigorous essence in man, regular menstruation in woman, combination of essence in woman and man contribute to pregnancy.” It emphasizes that harmonious thoroughfare vessel and conception vessel and gradual menstruation. The key to gestation lies in the maturation of eggs and the filling and strengthening of kidney essence.\(^\text{16,17}\) According to the *Comprehensive Classic of Chinese Medicine* (Yi Xue Zheng Zhan): “Since kidney water is insufficient, the menstrual blood is drying up day by day.” That is to say, kidney deficiency is the core pathogenesis of DOR. Bushen Culan prescription is a clinical empirical prescription, with the main effects of tonifying the liver and kidney, nourishing blood and regulating menstruation, and soothing the liver; Huangqi (Astragali Radix) in the prescription can tonify kidney qi; Shudihuang (Rehmanniae Radix Praeparata), Tusizi (Cuscutae Semen), Yinyanghuo (Epimedii Folium), Nyu Zhenzi (Ligustri Lucidi Fructus), Mohanlian (Ecliptae Herba) are used for warming yin and yang, nourishing yin and assisting yang to adapt to the dynamic changes of yin and yang and the requirement of yang growth of body at this stage; Baishao (Paoniae Alba Radix), Chaihu (Bupleuri Radix), and processed Xiangfu (Cyperi Rhizoma Praeparata) mainly exert the effects of softening and soothing the liver and relieving acuteness; Dansgui (Angelicae Sinensis Radix) and Chuanxiong (Chuanxiong Rhizoma) exert the effects of tonifying blood, harmonizing blood and regulating menstruation to meet the needs of ovulation induction and transformation promotion and promote the follicle to break through from the ovary; processed Gancao (Glycyrrhizae Radix et Rhizoma Praeparata cum Melle) is used to harmonize other herbs. All herbs are combined to exert the effects of tonifying the liver and kidney, soothing and softening the liver wood, nourishing blood, and regulating menstruation so that the essence and blood are sufficient and Tiangui reaches.\(^\text{18}\)

The decrease in oocyte production capacity or their quality in DOR patients will cause a decrease in the number of follicles remaining in the ovaries. Long-term infertility, if not treated in time, can further develop into premature ovarian failure, which will seriously affect the physiological and mental health of women. Although it can provide advantageous treatment by modern reproductive technology, this technology has not been completely accepted by patients.\(^\text{19}\)

In addition, with the decrease of oocyte quality, the stress response to drugs was also reduced. As a result, the search for safe and effective treatment is the current direction of the treatment of DOR. In modern medicine, it is believed that the occurrence of DOR can be caused by a variety of factors, such as multiple miscarriage history, infection, radiotherapy and chemotherapy, immune factors, poor living habits, psychological stress.\(^\text{20,21}\) Clinically, it is believed that the hypothalamic-pituitary-gonadal axis is the main pathway to control the menstrual cycle. Under the stimulation of hypothalamic gonadotropins, the pituitary gland releases FSH, which is also subject to the two-way feedback regulation of E2 and serum inhibin. The increase of FSH can lead to premature follicular formation in the body and shorten the menstrual cycle.\(^\text{22}\) Therefore, its expression level can indirectly evaluate the ovarian reserve function of the patients and reflect the degree of DOR development, but its variability is large and individual differences are obvious. It can only be used as a rough reference in clinical application, and there is no obvious range threshold. In clinical practice, FSH $\geq 10$ IU-L$^{-1}$ is often used as a predictive criterion for ovarian dysfunction.\(^\text{23}\) Studies have shown that the FSH/LH ratio can be used as an indicator to measure ovarian reserve function, that is, FSH/LH $> 3.6$ indicates a decrease in ovarian reserve function, and FSH/LH $> 2.0$ indicates early manifestations of DOR.\(^\text{24–26}\)

This study showed that the control group was treated with western medicine, and its clinical effect and pregnancy results were unsatisfactory. After a long time of regulation, if it fails to promote ovulation or pregnancy, the patients would enter the next treatment cycle again. The longer the waiting time, the mental and economic pressure of the patients would inevitably be increased, and a large amount of treatment time would be consumed, and for older women, the chances of conception would reduce again. The clinical efficiency, ovarian function improvement, sex hormone level, and pregnancy outcome of the observation group were better than those of the control group, suggesting that the combination regimen could actively improve the ovarian function of the patients, delay or even avoid the occurrence of premature ovarian failure, correct the abnormal levels of sex hormone (the basal hormone levels of FSH, LH, E2, and FSH/LH of patients after treatment were significantly improved compared with those before treatment), fully reflect the characteristics of syndrome differentiation and treatment of Chinese medicine prescriptions, and play a synergistic role with western medicine.

**Conclusion**

Bushen Culan prescription has definite effects on infertility with DOR. The mechanism may involve multicomponent and multitarget stimulation, which plays a role in improving the ovarian function of patients, regulating the levels of sex hormones, and thus improving pregnancy outcome.

**CRedit Authorship Contribution Statement**

**Yanqi Feng:** Conceptualization, funding acquisition, supervision, writing –original draft. **Sujuan Ma:** Investigation, methodology, validation, writing-review & editing.
Funding

None.

Conflict of Interest

The authors declare no conflict of interest.

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