Pakistan J. Zool., pp 1-4, 2022. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20210108180158

Short Communication

Effect of Congrong Tusi Pill on Ovarian Artery Blood Flow and Sex Hormones in Adjuvant Treatment of Ovarian Function Decline

Jianmin Liu[1], Jiancheng Zhang[2], Peijuan Wang[3,*] and Moslem Lari Najafi[4]

[1]Department of Obstetrics and Gynecology, Huai'an TCM Hospital Affiliated to Nanjing University of Chinese Medicine, Huaian 223001, China

[2]Department of Traditional Chinese Medicine, Lianshui County People's Hospital, Huaian 223400, China

[3]Department of Obstetrics and Gynecology, Jiangsu Province Academy of Traditional Chinese Medicine, Nanjing 210028, China

[4]Pharmaceutical Sciences and Cosmetic Products Research Center, Kerman University of Medical Sciences, Kerman, Iran

Jianmin Liu and Jiancheng Zhang contributed equal to this article as co-first author.

Article Information
Received 08 January 2021
Revised 01 March 2021
Accepted 18 April 2021
Available online 18 May 2022
(early access)

Authors' Contributions
JL arranged patients into groups while JZ followed up their physical conditions. PW made a comparative analysis of patients. MLN helped in the medical analysis. All authors conducted the experiments, analysed the results and wrote the manuscript.

Key words
Ovarian function decline, New addition congrong tusi pill, Ovarian artery blood flow, Sex hormones.

ABSTRACT

The present study was aimed to investigate the effect of new addition congrong tusi pill on ovarian artery blood flow and sex hormones in the adjuvant treatment of ovarian function decline. Ninety patients with ovarian function decline were randomly divided into 3 groups: new addition congrong tusi pill treatment group (n=30 cases), diethylstilbestrol treatment group (n=30 cases) and the control group (n=30 cases). A comparative analysis is made on sex hormone levels, ovarian artery blood flow, ovarian volume and number, treatment efficacy, glucose metabolism, ovulation and fertility in each group. There are statistical differences in sex hormone level, ovarian arterial blood flow, ovarian volume and number, treatment efficacy, glucose metabolism, ovulation and fertility in each group ($P<0.05$). The total effective rate of new addition congrong tusi pill is 100.00% for ovarian function decline, while that of diethylstilbestrol treatment is only 83.33%, and new addition congrong tusi pill group shows better recovery in each index compared to diethylstilbestrol, with patients' ovarian function greatly improved. To conclude, new addition congrong tusi pill has better clinical effect in improving ovarian artery blood flow and sex hormone levels than diethylstilbestrol in adjuvant treatment of ovarian function decline, which is worthy of clinical popularization and use.

Ovarian function decline means women once had a normal and natural menstrual cycle, but before the age of 45, there is ovarian atrophy-induced menstrual volume reduction, shortened menstrual period, and increased menstrual cycle until continued amenorrhea (Marongiu, 2019; Li *et al*., 2019). Clinical studies have found (Huang *et al*., 2019) that there are many reasons for ovarian function decline, including "natural" decline of ovarian function caused by age factors and pathological ovarian function decline caused by pathogenic factors such as radiotherapy, chemotherapy, surgery, *etc*. Menopause as a landmark event is essentially due to follicles exhaustion in the ovary which leads to greatly reduced ovarian estrogen level and therefore occurrence and development of ovarian diseases (Behmanesh *et al*., 2019). The process of ovarian function decline is a gradually accumulated complex biological process under regulation of multiple factors, which concerns complex endocrine and metabolic diseases in multiple disciplines and fields, such as abnormal sex hormone levels and ovarian arterial blood flow (Trabert *et al*., 2019). In this study, 90 patients with ovarian function decline were enrolled to investigate the effect of new addition congrong tusi pill on ovarian artery blood flow and sex hormones in adjuvant treatment of ovarian function decline.

* Corresponding author: wangpeijuan999@163.com
0030-9923/2022/0001-0001 $ 9.00/0

Martials and methods

From February 2015 to December 2019, 90 patients diagnosed with ovarian function decline at the

Reproductive Endocrinology Clinic of the Municipal Central Hospital were enrolled in the study, whose average age was 27.24±5.18 years.

Inclusion criteria: (i) The patients meet the 2003 Rotterdam ovarian function decline diagnostic criteria; (ii) The patients have oligomenorrhea or amenorrhea; (iii) Transvaginal sonography or pelvic ultrasonography finds that the patient has ≥12 follicles with ovarian diameter at 2-9 mm on at least one side, or the ovarian volume≥ 10 mL; (iv) The patient's clinical data is complete and accurate, and there are no other diseases; (v) All study subjects have signed informed consent.

Exclusion criteria: (i) With other endocrine diseases that cause excessive androgen and ovulation disorders, such as congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumors, hyperprolactinemia, thyroid diseases, *etc*.; (ii) Patients who cannot cooperatively complete this experiment due to various reasons. Ninety patients with ovarian function decline were randomly divided into 3 groups: new addition congrong tusi pill treatment group (n=30 cases), diethylstilbestrol treatment group (n=30 cases) and the control group (n=30 cases). New addition congrong tusi pill treatment (6): Rehmannia glutinosa and Angelica sinensis 10g each, Cistanche, Cuscuta, Raspberry, Herba Taxilli, motherwort fruit, Cornus, herba lycopi, Morinda citrifolia, Epimedium, sauteed cyperus rotundus 15 g each. Add 100 mL water each time and decoc, administer 3 times a day orally, 1 dose in 2 days, a total of 7 doses. Attention should be paid to rest, regular work and rest, and nutrition enhancement in all aspects. Diethylstilbestrol treatment: administer diethylstilbestrol 0.25-1.0 mg/day from the 6th day of the patient's routine cycle for 20 days, and add medroxyprogesterone acetate 10 mg/day on the 16-25th day of the cycle, or from the 17th day in the cycle, intramuscularly inject 20 mg of progesterone every other day for 5 times. For control group the patient was allowed to recover naturally without any medication.

Before and after treatment, cubital venous blood was drawn on empty stomach between 8:00 and 10:00 in the morning on the 2-5 days of the patient's menstrual cycle (first determine the non-dominant follicles in the bilateral ovaries in the case of amenorrhea). After centrifugation for 10 min under 3500r/min, the serum was collected and stored in a refrigerator at -20°C. According to the relevant kit instructions, the serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (TT), estrogen, sex hormone binding globulin (SHBG) and dehydroepiandrosterone sulfate (DHEAS) levels were determined by chemiluminescence method in each group. The measuring instrument sensitivity was set to 1.1 pg/mL, the coefficient of variation (CV) was ≤10%, and each sample was measured 3 times (Moini *et al*., 2019). Before and after the treatment, transvaginal sonography (TVS) was performed: the patients took supine position and all were first checked by conventional ultrasound: the vaginal ultrasonic probe frequency was maintained at 6.0-10.0 MHz. Patient's interstitial artery peak flow velocity (Vmax), pulsatility index (PI), and blood flow resistance index (RI) (He *et al*., 2020). The follicular number (FN), ovarian volume (OV), ovarian stroma area (SA), and ovarian stroma area/total area (SA/TA) were recorded.

Treatment efficacy consists of cured, markedly effective, effective and ineffective. Where, cured means the patient's menstruation has returned to a normal cycle, and other symptoms have basically disappeared, the patient can maintain 3 or more normal menstrual cycles or have become pregnant after the treatment is stopped. Markedly effective means the patient's menstruation has basically returned to a normal cycle (28±7 d) after treatment, and other symptoms have basically disappeared or alleviated. Effective means the menstrual cycle is improved after treatment, and after treatment is stopped, other symptoms are alleviated compared with before. Ineffective means that menstruation is still not seen after 3 months of continuous treatment, and other symptoms have not been relieved. The total effective rate of treatment is calculated based on the above results.

Before and after treatment, the serum of each group was taken, and fasting blood-glucose (FBG) was detected by automatic biochemical analyzer, and the insulin (INS) was measured with the kit. The homeostasis model assessment of insulin resistance (HOMA-IR) was performed at the same time. HOMA-IR≥2.69 indicates insulin resistance (Sozen *et al*., 2019).

All patients underwent ultrasonography during the follicular phase before and after treatment. Any date is possible in the case of amenorrhea. Pelvic ultrasonography was performed transvaginally (through the anus for unmarried individuals) to record the patient's cystic dilated follicle rate, average ovulation number and ovulation rate. SPSS 20.0 statistical analysis software was used.Measurement data is expressed as "mean ± standard deviation" ($\bar{x}$±s), and independent sample t test is used for comparison. Count data is expressed as percentage (%), and comparison is made by χ^2 analysis. P<0.05 indicates statistically significant difference.

Results and discussion

Before treatment, there is no significant difference in the levels of various sex hormones between the two groups (P>0.05). However, treatment group shows superior result than the diethylstilbestrol group, and comparison between the groups is statistically significant (P<0.05); the levels of sex hormones FSH, LH, Estrogen, SHBG and DHEAS increase after treatment, showing statistically significant

Table I.- Sex hormone levels, ovarian artery blood flow, ovarian volume, glucose metabolism, ovulation, fertility and number in each group ($\bar{x}\pm s$).

Group	Control group	New addition congrong tusi pill group		Diethylstilbestrol group	
		Before treatment	After treatment	Before treatment	After treatment
FSH (mIU/mL)	6.21±1.13	4.29±1.27	6.33±1.53	4.53±2.01	5.11±1.02
LH (mIU/mL)	4.83±1.94	10.77±3.31	5.08±2.65	10.53±3.15	7.75±2.11
TT (ng/mL)	0.39±0.12	0.76±0.24	0.40±0.14	0.82±0.12	0.60±0.21
Estrogen	148.34±21.09	137.58±84.10	146.41±98.64	137.92±54.23	140.21±34.12
SHBG (nmol/L)	136.23±21.43	77.86±6.78	134.63±10.73	77.90±11.65	100.92±11.43
DHEAS	176.03±39.02	253.75±14.47	182.39±12.35	253.11±15.03	212.43±30.12
Vmax (cm/s)	8.01±1.64	12.32±4.32	8.19±2.23	12.54±2.54	9.63±2.32
PI	1.87±0.91	1.42±0.76	1.99±0.87	1.39±0.34	1.64±0.73
RI	0.78±0.23	0.53±0.12	0.76±0.09	0.59±0.11	0.60±0.14
FN	5.89±2.44	16.05±3.01	6.15±1.24	16.13±2.65	8.23±3.35
OV (mL)	6.01±2.13	14.16±2.87	6.31±1.42	14.23±3.21	9.24±2.76
SA (cm2)	1.14±0.97	2.56±0.98	1.22±0.34	2.75±0.87	2.09±1.01
TA (cm2)	4.95±1.26	5.56±1.24	5.09±1.09	5.43±2.33	5.35±2.83
SA/TA	0.22±0.09	0.46±0.11	0.24±0.09	0.53±0.12	0.38±0.14
FBG (mmol/L)	5.43±2.21	5.41±1.23	5.79±1.73	5.52±2.24	5.56±1.98
INS (μIU/mL)	10.98±3.49	14.55±2.43	10.32±0.98	15.02±4.12	12.30±3.21
HOMA-IR	3.98±1.02	3.91±0.76	2.63±0.04	3.94±2.03	3.81±1.82
Cystic dilated follicle rate (%)	0.24±0.12	34.32±2.65	0.03±0.00	36.12±3.49	10.92±3.21
Average ovulation No.	3.31±1.03	12.87±0.98	3.76±0.43	13.42±4.12	5.01±2.29
Ovulation rate (%)	99.92±9.92	62.31±4.98	99.75±6.02	63.76±8.33	82.63±6.71

FSH, serum follicle-stimulating hormone; LH, luteinizing hormone; TT, total testosterone; SHBG, sex hormone binding globulin; Vmax, flow velocity; PI, pulsatility index; FN, follicular number; OV, ovarian volume; SA, ovarian stroma area; TA, total area; FBG, fasting blood-glucose; INS, insulin; HOMA-IR, homeostasis model assessment of insulin resistance.

difference (P<0.05). Vmax decreases after treatment. However, new addition congrong tusi pill treatment group shows superior result than the diethylstilbestrol group, and comparison between the groups is statistically significant (P<0.05); while PI and RI increase after treatment, showing statistically significant difference (P<0.05). Before treatment, there is no significant difference in ovarian volume and number between the two groups (P>0.05). After treatment, FN, OV, SA, TA, and SA/TA are all reduced, but new addition congrong tusi pill treatment group shows superior result than the diethylstilbestrol group, and comparison between the groups is statistically significant (P<0.05) (Table I). Before treatment, there is no significant difference in glucose metabolism between the two groups (P>0.05). After treatment, both INS and HOMA-IR decrease, and FBG increases significantly. The new addition congrong tusi pill treatment group shows superior result than the diethylstilbestrol group, and comparison between the groups is statistically significant (P<0.05). Before treatment, there is no significant difference in ovulation and fertility between the two groups (P>0.05). After treatment, the cystic dilated follicle rate and the average ovulation number decrease, and the ovulation rate increases significantly. The new addition congrong tusi pill treatment group shows superior result than the diethylstilbestrol group, and comparison between the groups is statistically significant (P<0.05) (Table I). The proportions of cured, markedly effective, effective and ineffective cases in the new addition congrong tusi pill group after treatment are 76.67%, 20.00%, 3.33%, and 0.00%, respectively, and the total effective rate is 100.00%; The proportions of cured, markedly effective, effective and ineffective cases in the diethylstilbestrol group after treatment are 46.67%, 16.67%, 20.00% and 16.67%, respectively, and the total effective rate is 83.33%. In comparison of treatment efficacy between the two groups, the new addition congrong tusi pill group is superior to the diethylstilbestrol group, showing statistically significant difference (P<0.05) (Table II).

In the treatment of ovarian function decline, new addition congrong tusi pill is mainly to nourish the kidney and qi, invigorate the kidney and essence replenishment, harmonize chong and ren channels, warm the kidney and uterus. Where, Cuscuta and Fructus Lycii are mainly used to warm the kidney, replenish essence and blood. Raspberries are sweet and sour and have a good effect of supplementing the kidney to control the nocturnal. Cistanche has the function of warming the kidney and enhancing yang to boost junior fire. Rehmannia glutinosa nourishes blood and yin, replenishes vital essence and

nourishes the bone marrow. Angelica can nourish blood and promote blood circulation. Herba Taxilli can reinforce the liver and kidney and strengthen the bones and muscles (Abdelnaby *et al*., 2020; Chen *et al*., 2020). Clinical research experiments have shown (Liu *et al*., 2019) that excessive androgen in the ovaries will also inhibit follicular maturation, thereby blocking further follicular development, and hindering normal formation of high-quality follicles. High levels of male hormones may also directly or indirectly cause abnormal glucose metabolism in the body and form insulin resistance. Such high levels of male hormones and insulin resistance will form a vicious circle, which affects the normal follicular development (Sheshpari *et al*., 2019). The pituitary gland of patients with ovarian function decline has increased sensitivity to changes in the level of hormones released by hypothalamic gonadotropin, leading to an increased level of luteinizing hormone, while excessive luteinizing hormone stimulates more secretion of male hormones by follicular stroma and follicular theca cells (Elkady *et al*., 2019).

Table II.- Analysis of treatment efficacy in two groups (n (%)).

Group	New addition congrong tusi pill group	Diethylstil-bestrol group	χ^2	P value
n	30	30	-	-
Cured	23(76.67)	14(46.67)	9.231	0.002
Markedly effective	6(20.00)	5(16.66)	3.823	0.041
Effective	1(3.33)	6(20.00)	17.884	0.002
Ineffective	0(0.00)	5(16.67)	16.331	0.003
Total effective rate (%)	73(100.00)	59(83.33)	12.253	0.003

Our results indicate that blood perfusion in the ovaries of patients with ovarian decline encounters low resistance, and the occurrence of this phenomenon has relation to the increased migration ability of new vascular endothelial cells in the ovary, which further promotes the formation of new blood vessels (Han *et al*., 2019).

Based on the above studies, it is shown that new addition congrong tusi pill has high application value in improving ovarian arterial blood flow and sex hormone levels in adjuvant treatment of ovarian function decline, which can provide scientific theoretical basis for the treatment of ovarian function decline and formulation of corresponding treatment plan, thus laying a solid foundation for the further study of the follow-up clinical trials of the disease.

Statement of conflict of interest

The authors have declared no conflict of interests.

References

Abdelnaby, E.A., Abo El-Maaty, A.M. and El-Badry, D.A., 2020. *Reprod. Domest. Anim.,* **55**: 701-710. https://doi.org/10.1111/rda.13668

Behmanesh, N., Abedelahi, A., Charoudeh, H.N. and Alihemmati, A., 2019. *Turkish J. Obstet. Gynecol.,* **16**: 143. https://doi.org/10.4274/tjod.galenos.2019.46244

Chen, W.H., Huang, K.H. and Kung, F.T., 2020. *J. Obstet. Gynaecol. Res.,* **46**: 752-758. https://doi.org/10.1111/jog.14236

Elkady, M., Shalaby, S., Fathi, F. and El-Mandouh, S., 2019. *Human exp. Toxicol.,* **38**: 1283-1295. https://doi.org/10.1177/0960327119865588

Han, M., Cheng, H., Wang, J., Yu, Y., Wang, F., Zhu, R., Wang, W., Yang, S. and Li, H., 2019. *Gynecol. Endocrinol.,* **35**: 985-990. https://doi.org/10.1080/09513590.2019.1616173

He, Y., Wang, Q., Li, X., Wang, G., Zhao, J., Zhang, H. and Chen, W., 2020. *Fd. Funct.,* **11**: 5192-5204. https://doi.org/10.1039/C9FO02554E

Huang, B., Qian, C., Ding, C., Meng, Q., Zou, Q. and Li, H., 2019. *Stem Cell Res. Ther.,* **10**: 1-12. https://doi.org/10.1186/s13287-019-1317-7

Li, Q., An, X., Man, X., Chu, M., Zhao, T., Yu, H. and Li, Z., 2019. *Life Sci.,* **239**: 116999. https://doi.org/10.1016/j.lfs.2019.116999

Liu, R., Zhang, X., Fan, Z., Wang, Y., Yao, G., Wan, X., Liu, Z., Yang, B. and Yu, L., 2019. *Stem Cell Res. Ther.,* **10**: 1-14. https://doi.org/10.1186/s13287-019-1315-9

Marongiu, R., 2019. *Front. Aging Neurosci.,* **11**: 242. https://doi.org/10.3389/fnagi.2019.00242

Moini, A., Pirjani, R., Rabiei, M., Nurzadeh, M., Sepidarkish, M., Hosseini, R. and Hosseini, L., 2019. *J. Ovarian Res.,* **12**: 1-7. https://doi.org/10.1186/s13048-019-0551-z

Sheshpari, S., Shahnazi, M., Mobarak, H., Ahmadian, S., Bedate, A.M., Nariman-Saleh-Fam, Z., Nouri, M., Rahbarghazi, R. and Mahdipour, M., 2019. *J. Translat. Med.,* **17**: 1-15. https://doi.org/10.1186/s12967-019-02149-2

Sozen, B., Ozekinci, M., Erman, M., Gunduz, T., Demir, N. and Akouri, R., 2019. *J. Assisted Reprod. Genet.,* **36**: 2181-2189. https://doi.org/10.1007/s10815-019-01560-4

Trabert, B., Coburn, S.B., Falk, R.T., Manson, J.E., Brinton, L.A., Gass, M.L., Kuller, L.H., Rohan, T.E., Pfeiffer, R.M. and Qi, L., 2019. *Cancer Causes Contr.,* **30**: 1201-1211. https://doi.org/10.1007/s10552-019-01233-8