ORIGINAL ARTICLE

The effect of acupuncture on anti-mullerian hormone and assisted reproduction outcome in Polycystic Ovary Syndrome patients undergoing in vitro fertilization

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Abstract

Objectives: To evaluate the effect of acupuncture at follicular phase of menstrual cycle on anti-mullerian hormone levels in patients with polycystic ovary syndrome undergoing in-vitro fertilisation and to see its impact on assisted reproduction outcome.

Methods: The prospective, randomised, controlled trial was conducted from March 2011 to July 2012 at the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. In the center, the patients randomly chose odd or pair number, the patients with odd numbers classified as an interventional group and the patients with paired numbers as non-interventional group. Infertile polycystic ovary syndrome patients aged 20-40 years were enrolled from the hospital's Assisted Reproduction Centre from March 2011 to July 2012. The patients were randomised into two groups, with one receiving follicular phase acupuncture for 30-40 minutes according to the principles of traditional Chinese medicine, and the other group not getting subjected to acupuncture. Serum and follicular anti-mullerian hormone concentration were determined.

Results: Of the 102 patients, 33(32.4%) were in the intervention group, while 69(67.6%) were in the control group. There was no significant effect of acupuncture on serum and follicular fluid anti-mullerian hormone levels in the intervention group compared to the control group (p>0.05). Serum progesterone and estradiol levels on the day of giving human chorionic gonadotrophin, as well as serum progesterone and estradiol levels on the day of oocytes pick-up were significantly lower in the intervention group (p<0.05). Number of embryos transferred, clinical and ongoing pregnancy rates were significantly higher in the intervention group (p<0.05) with a significant decrease of ovarian hyper-stimulation syndrome rate in the intervention group (p<0.05).

Conclusions: Follicular phase acupuncture was found to have a positive effect for polycystic ovary syndrome patients undergoing in-vitro fertilisation, but it had no effect on anti-mullerian hormone concentrations.

Keywords: Acupuncture, Anti-mullerian hormone, Polycystic ovary syndrome. (JPMA 69: S-4 (Suppl. 3); 2019)

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous collection of singns and symptoms that form a spectrum of disorders that has a mild presentation in some cases, and a severe disturbance of the reproductive, metabolic and endocrine functions in others. Key features include menstrual cycle disturbance, hyper-androgenism and obesity.[1] Obesity-associated reproductive and metabolic dysfunctions may aggravate the symptoms of PCOS.[2] It is the most common cause of anovulatory infertility in women of reproductive age, affecting ~7% of this particular population segment.[3] Although PCOS patients typically produce increased number of oocytes, but they are often of poor quality, leading to lower fertilisation, cleavage and implantation rates, and a higher miscarriage rate.[4-6] Anti-mullerian hormone (AMH) is a glycoprotein

dimeric hormone that belongs to the transforming growth factor beta (TGF-β) super-family.[7] The highest level of AMH expression is seen in the granulosa cells of secondary, preantral, and small antral follicles no more than 4mm in diameter, and the expression disappears as follicles develop to the larger antral stage.[8] AMH concentrations are supra-physiological in those with an excess of small antral follicles, classically in patients with PCOS.[9] The main physiological role of AMH in the ovary seems to be limited to the inhibition of the early stages of follicular development.[10] The pathogenesis of PCOS remains largely unknown although recent studies have suggested that AMH may have a role to play in the ovarian follicular status in PCOS.[11] In anovulatory patients with PCOS, granulosa cell function is abnormal, therefore, the abnormality of granulosa cells in PCOS may influence oocyte or embryo quality.[12] In vitro fertilisation (IVF) is a major treatment in infertility. In the year 2000, about 200,000 babies were conceived throughout IVF worldwide.[13] Acupuncture is a popular treatment choice for infertility.[14] It is an ancient traditional Chinese

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treatment with an empirical basis originating 2500 years ago. It is one of the most widespread forms of complementary and alternative medicine (CAM) in the United States and Europe.[15] Paulus et al. evaluated the effect of acupuncture on IVF pregnancy rate by comparing a group of patients who underwent acupuncture treatment shortly before and after embryo transfer (ET) with the control group which did not have acupuncture.[16] The theory of acupuncture is based on the fact that disruptions of natural balanced energy flow (Qi) are responsible for the pathogenesis of the disease. Randomised controlled trials (RCTs) evaluated the effect of acupuncture on reproductive outcome in patients treated with IVF/intra cytoplasmic sperm injection (ICSI).[17,18] A more recent study showed that acupuncture improves clinical pregnancy rates and live-birth rates among women undergoing IVF.[19]

The current study was calculated to evaluate the effect of acupuncture at the follicular phase of menstrual cycle on AMH levels in PCOS patients undergoing IVF, and to see its impact on assisted reproduction outcome.

Patients and Methods

The prospective RCT study was conducted from March 2011 to July 2012 at the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The study was approved by the institutional ethics committee of Tongji Medical College of Huazhong University of Science and Technology and the trial have been registered in the ClinicalTrial.govIdentifier: NCT01778621. Patients were enrolled from the hospital's Assisted Reproduction Centre and written informed consent was obtained from each of them. The diagnosis of PCOS was made on the basis of the presence of two out of three conditions: amenorrhoea or oligomenorrhoea, polycystic ovaries, and hyper-androgenism.

Those included were PCOS patients aged 20-40 years with normal uterine cavity as evaluated by hysterosalpingograph, patent one or two fallopian tubes, and normal semen analysis for patient's partner.

Those excluded were patients with disorders that have similar clinical presentation, like Cushing's syndrome, congenital adrenal hyperplasia, thyroid dysfunction and hyperprolactinaemia, as well as closed both tubes and abnormal semen analysis for patient's partner.

All patients were down-regulated according to the long protocol[20] adopted in Assisted Reproduction Center in Union Hospital as they received a standard gonadotrophin-releasing hormone (GnRH) agonist daily

injection regimen on day 21 of the preceding menstrual cycle until the day of giving human chorionic gonadotrophin (HCG) injection. Pituitary and ovarian suppression was confirmed by a plasma follicular stimulating hormone (FSH), leutinizing hormone (LH) levels <5mIU/ml, plasma estradiol (E2) level <50pg/ml and/or endometrial thickness <5 mm. Ovarian stimulation was performed using recombinant human follicle-stimulating hormone (r-hFSH) Follitropin Alpha (GONAL-f®) 75 -150IU on the third day of subsequent withdrawal bleeding and the dose was determined according to patient's age and the count of antral follicles estimated by trans-vaginal ultrasound (TVU) after which ovulation was triggered using single injection of HCG 10,000IU intra-muscularly (IM) when at least three follicles had a diameter of more than or equal to 18mm with an adequate serum E2 concentration.

Oocyte retrieval was performed 36h later and then they were inseminated. Fertilisation was checked 18h later and then morphologically top-quality embryos were judged to be transferred into the patient´s uterus on day 3 after retrieval. All patients received luteal-phase support. Biochemical pregnancy was established depending on serum β-HCG concentration on day 14 after ET.

Acupuncture was performed starting from the third day of the cycle together with IVF protocol and was continued daily till the day of giving HCG. For the purpose, the patients were randomised into intervention and control groups. Acupuncture was applied by using 4cm long stainless steel hair-thin needles. Needle reaction, like soreness, feeling of heaviness that distend around the site of acupuncture or sometimes extend along the corresponding meridians (called the DeQi sensation), was felt during the initial insertion. The needles remained for 30-40 min and were then removed. In this study and according to the principle of traditional Chinese medicine, the following acupuncture points were used; Tai chong (TCM) (LIV 3), San yin jiao (SP6), Diji (SP 8), Zu san li (ST 36), Xuehai (SP 10), Guilai (ST 29), Hegu (LI 14), and Guan yuan (Ren 04).

Accurate concentration of follicular-fluid AMH was obtained by collecting the follicular fluid from the first retrieved follicle from both ovaries. A total of 204 follicular fluid samples that were collected from the patients were analysed to obtain the average AMH concentration. On the morning prior to oocyte retrieval, blood samples were obtained from all the patients. Serum and follicular AMH concentration were determined using Enzyme Linked Immunosorbent Assay Kit for AMH (Uscn, Life Science Inc., Wuhan 430056, P.R. China). The minimum detectable concentration of AMH

is <0.058ng/ml with intra-assay coefficient of variation (CV)<10% and inter-assay CV<12% and the results were expressed as ng/ml. Both E2 and progesterone hormones level were measured using the ELFA technique, BIOMERIEUX, France). E2 analytical detection limit is 9pg/ml with a probability of 95% and for progesterone equal to 0.25ng/ml with a probability of 95%.

Statistical Analysis

Data analysis was performed by using Statistical Package for Social Sciences (SPSS, version 19). We used Student's t-test and Chi-square to assess for differences in independent variables at baseline between interventional and non-interventional groups. The two-tailed P-value less than 0.05 was considered statistically significant.

Results

Of the 102 patients, 33(32.4%) were in the intervention group, while 69(67.6%) were in the control group. There

Table-1: Schedules of in-vitro fertilisation.

	Interventional n=33	Non-interventional n=69	2-tailed P-value*
Pituitary down regulation (ds)	20.39±1.45	20.26±1.26	0.638
Ovarian stimulation duration (ds)	9.45±1.34	9.72±1.44	0.369
GnRH ampules	10.23±1.00	10.25±1.77	0.944
hFSH ampules	19.72±5.31	21.50±6.30	0.165

Data are presented as mean ± standard deviation (SD).
GnRH: Gonadotrophin-releasing hormone.
hFSH: Human follicle-stimulating hormone.

was no statistical difference regarding number of GnRH and hFSH ampoules that were used in the stimulation protocol and the duration of ovarian stimulation between the two groups (p>0.05) (Table-1).

Also, there was no statistical difference between the groups in terms of the number of oocytes retrieved (p>0.05), but there was a significant difference between

Table-2: Characteristics of ovarian response in women with polycystic ovarian yndrome (PCOS) (intervention and control groups).

	Interventional n=33	Non-interventional n=69	2-tailed P-value*
No. of >14mm follicle	15.33 ±7.84	16.71± 5.59	0.312
No. of oocytes retrieved	23.72 ±12.34	26.28 ±11.57	0.308
No. of MII oocytes	18.60 ±9.69	22.31± 8.97	0.06
No. of II PN	12.81± 6.89	15.59 ±7.05	0.064
Serum estradiol (pg/ml) on HCG day	8678.38±3865	10011.62±2705	0.046
Serum estradiol (pg/ml) on dOPU.	5158.30±3712	7059.01±3446	0.013
Serum progesterone (ng/ml) on HCG day	1.15 ± 0.33	1.45±0.57	0.007
Serum progesterone(ng/ml) on dOPU	12.02 ± 8.39	15.97±9.47	0.044
Serum AMH (ng/ml)	3.68±1.25	3.59±1.37	0.769
Follicular fluid AMH (ng/ml)	7.46±2.36	7.84±2.10	0.408
Endometrial thickness (mm)	10.48 ± 1.98	10.39±2.05	0.828
Fertilization rate (%)	66.41±18.25	71.65±11.70	0.139
Implantation rate (%)	29.68±18.68	7.97±20.33	0.035

Data are presented as mean ± SD. CI: Confidence interval; M II=metaphase II, AMH=anti-Mullerian hormone, dOPU=day of oocyte pick-up, HCG=human chorionic gonadotrophin, 2PN=pro nuclei.

Table-3: Outcome of in-vitro fertilisation technique.

	Interventional n=33	Non-interventional n=69	OR	95%CI	2-tailed P-value*
Embryo transferred patients (no.)	(19/33) 57.60%	(21/69) 30.40%	3.102	1.313-7.330	0.009
Biochemical pregnancy rate	(14/33) 42.40%	(15/69) 21.70%	2.653	1.082-6.502	0.03
Clinical pregnancy rate	(11/33) 33.30%	(11/69)15.90%	0.379	0.144-1.000	0.046
Ongoing pregnancy rate	(11/33) 33.30%	(10/69)14.50%	0.339	0.126-0.909	0.028
OHSS	(12/33) 36.40%	(45/69) 65.20%	0.305	0.128-0.724	0.006
Thin endometrium	(1/33) 3.00%	(2/69) 2.90%	1.047	0.092-11.976	0.971
High progesterone level	(0/33) 0%	(1/69) 1.40%	1.015	0.986-1.044	0.487
No fertilization	(1/33) 3.00%	(0/69) 0%	0.97	0.913-1.030	0.146
Ectopic pregnancy and abortion	(0/33) 0%	(2/69) 2.90%	1.03	0.989-1.073	0.323

Data are presented as numbers (%). OHSS=ovarian hyper stimulation syndrome, OR=odds ratio.

serum E2 and progesterone on day of oocyte pick-up (dOPU) p<0.05) and serum E2 on the day of giving HCG (p=0.046). Progesterone on HCG day was significantly lower than the control group (p=0.007). No statistical significance regarding serum AMH and follicular fluid AMH was found between the groups (p>0.05) (Table-2).

The number of ET patients as well as clinical pregnancy and ongoing pregnancy rates were significantly different (p<0.05). The most serious complication of the ovarian stimulation protocol that delayed ET was ovarian hyper stimulation syndrome (OHSS) which was significantly low in the interventional group (p=0.006) (Table-3).

Discussion

Many PCOS patients need prolonged treatment. One study showed that acupuncture causes a specified pattern of afferent activity in peripheral nerves. It affects the hypothalamic-pituitary-adrenal (HPA) axis by decreasing cortisol concentrations[21] and the hypothalamic-pituitary-gonadal (HPG) axis by modulating central and peripheral B-endorphin production and secretion, thereby influencing the release of hypothalamic GnRH and pituitary secretion of gonadotrophin.[22-24] On the other hand, the positive effect of acupuncture during IVF treatment may be related to the changes in uterine-uterine contractility, blood flow and relaxation of stress[25] which means it affects PCOS symptom by modifying endogenous regulatory systems. The changes are most likely mediated via the endogenous opioid system.[21,24]

The study showed no effect on AMH concentrations perhaps due to small sample size of the study or the role of acupuncture for the treatment of PCOS patients had no effect on AMH concentration. AMH concentration in serum was lower than that for follicular fluid which either due to the fact that serum AMH concentration declined progressively during ovarian stimulation when using GnRH agonist protocol as has been reported by some studies published or perhaps circulating AMH concentrations reflected the growing follicles on the dOPU and were less effective in discriminating the per-follicle production of AMH, especially if the blood samples were collected in the follicular phase.[26,27]

Acupuncture has been shown to regulate fertility hormones as noted in this study because stress and other factors can disrupt the function of hypothalamic-pituitary-ovarian axis causing hormonal imbalances that can negatively impact fertility. Acupuncture has been shown to affect hormone levels by promoting the release of B-endorphin in the brain, which affects the release of GnRH

by the hypothalamus, FSH from pituitary gland and estrogen and progesterone levels from the ovary.[28] As such, acupuncture affects both ovaries by changing the pathogenesis of PCOS or modulates the hormonal effect on ovaries in a role of restoring the normal ovarian physiology. The acupuncture treatment can be applied for all PCOS patients seeking fertility in combination with medical drugs or in combination with IVF protocol.

The significantly better results regarding number of ET patients as well as clinical and ongoing pregnancy rates in the intervention group in the current study explains the positive effect of acupuncture in increasing blood flow to the uterus, improving the thickness of endometrium and increasing the chance of implantation. OHSS was one of the most serious complications that delayed ET. Prospective studies performed on a large number of subjects have shown the relevant value for AMH for the prediction of OHSS and hyper-response. The reported cut-off value is 3.5ng/ml, and beyond that OHSS and hyper-response may be expected.[29]

Conclusion

Study findings supported the significance of acupuncture in enhancing fertility among PCOS patients through increasing clinical and ongoing pregnancy rates, regulating fertility hormones and decreasing OHSS with no effect on AMH concentrations in follicular fluid and serum.

Conflicts of Interest: None

Disclaimer: None

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