The stress and distress of infertility: Does spirituality help women cope?

Alice D. Domar, PhD, Alan Penzias, MD, Jeffery A. Dusek, PhD, Amora Magna, BA, Dalia Merarim, PhD, Barbara Nielsen, MDiv, PhD, Debika Paul, BA

In this evaluation of nearly 200 infertile women, high levels of religiosity and spirituality are significantly correlated with low levels of psychological distress. Clinicians should be prepared to discuss religious and spiritual issues with their patients, as those issues may play an important role in the psychological health of infertile women—and in their response to infertility treatment.
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A Randomized Study Evaluating Acupuncture as an Adjunct to IVF

Source: Rodolfo Quintero, M.D., Wendy Yu, L.Ac., Brandon Horn, L.Ac., J.D., Daoshing Ni, D.O.M., Barry Schifrin, MD., Brian Acacio, M.D.
Department of Obstetrics and Gynecology, Glendale Adventist Medical Center (GAMC) and the Eastern Center for Complementary Medicine, Glendale, CA

A Randomized Study Evaluating Acupuncture as an Adjunct to IVF Acupuncture is based on patterns of energy flow (Qi) through a network of meridians. Maintenance of Qi is essential for health, while blockage may provoke disease. Acupuncture works by unblocking the various types of stagnation that can occur along these channels. After Paulus et. al demonstrated the benefit of acupuncture to IVF (Fertil Steril Apr 2002),
many IVF centers began offering these services.
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Reduction of blood flow impedance in the uterine arteries of infertile women with electro-acupuncture

Source: European Society for Human Reproduction and EmbryologySource: European Society for Human Reproduction and Embryology

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Acupuncture Normalizes Dysfunction of Hypothalamic-Pituitary-Ovarian Axis

Bo-Ying Chen M.D.
Professor of Neurobiology

This article summarizes the studies of the mechanism of electroacupuncture (EA) in the regulation of the abnormal function of hypothalamic pituitary-ovarian...
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Acupuncture Treatment For Infertile Women Undergoing Intracytoplasmic Sperm Injection

Sandra L. Emmons, MD
Phillip Patton, MD

Infertility is an area of women's health that has sparked much consumer interest in acupuncture. However, there is little published information concerning the combination of...
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Effects of Electro-Acupuncture on Nerve Growth Factor and Ovarian Morphology in Rats with Experimentally Induced Polycystic Ovaries

Elisabet Stener-Victorin,[2,3] Thomas Lundeberg,[4] Urban Waldenström,[3] Luigi Manni,[5] Luigi Aloe,[5] Stefan Gunnarsson,[6] and Per Olof Janson[3]

Despite extensive research on the pathogenesis of polycystic ovary syndrome (PCOS), there is still disagreement on the underlying mechanisms. The rat model for experimentally...
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Substitution of Acupuncture for HCG in Ovulation Induction

Cai Xuefen
Obstetrical & Gynecological Hospital,
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By using human menopausal gonadotropin (HMG) and human chorionic gonadotropin (HCG), fairly good clinical therapeutic efficacy has been obtained in the treatment of infertility...
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Relationship Between Blood Radioimmunoreactive Beta-Endorphin and Hand Skin Temperature During The Electro-Acupuncture Induction of Ovulation

Chen Bo Ying M.D. Lecturer of Neurobiology
Institute of Acupuncture Research,
and
Yu Jin, MD., Prof of Gynecology
Obstetricus and Gynecology Hospital
Shanghai Medical University
Shanghai, People's Republic of China

Thirteen cycles of anovulation menstruation in 11 cases were treated with Electro-Acupuncture (EA) ovulation induction. In 6 of these cycles...
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Influence of acupuncture on the pregnancy rate in patients who undergo assisted reproduction therapy

Wolfgang E. Paulus, M.D.,[a] Mingmin Zhang, M.D.,[b] Erwin Strehler, M.D.,[a]
Imam El-Danasouri, Ph.D.,[a] and Karl Sterzik, M.D.[a]

Acupuncture is an important element of traditional Chinese medicine (TCM), which can be traced back for at least 4,000 years.
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Role of acupuncture in the treatment of female infertility

By

Raymond Chang, M.D.[a,b] Pak H. Chung, M.D.[b] and Zev Rosenwaks, M.D.[c]

Although the understanding of acupuncture is based on ancient medical theory, studies have suggested that certain effects of...
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Clinical Studies of Chiropractic during Pregnancy

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Clinical Studies of Acupunture for Fertility and Pregnancy

Acupuncture has numerous potential fertility-boosting benefits according to New York Weill Cornell physician-scientists

http://www.news.cornell.edu/releases/April03/fertility.html

New York, NY (April 29, 2003)--Physician-scientists at the Center for Reproductive Medicine and Infertility (CRMI) at New York Weill Cornell medical center call for a definitive study of acupuncture as a fertility treatment, citing its numerous, promising benefits associated with increasing fertility in women. .An article in a recent issue of Fertility and Sterility--co-authored by Dr. Zev Rosenwaks, Dr. Pak H. Chung, and Dr. Raymond Chang of Weill Cornell--provides a summary of current research that supports acupunctures potential benefits for fertility treatment, including the stimulation of increased uterine blood flow and fertility hormones.

"Acupuncture, which is nontoxic and relatively affordable, holds much promise as a complementary or alternative fertility treatment," said Dr. Raymond Chang of New York Weill Cornell Medical Center. "Yet, while there are a great number of biological explanations for acupunctures benefits to fertility, as well as significant anecdotal evidence, there has yet to be a definitive clinical study," added Dr. Rosenwaks, Director of CRMI.

"One of the biggest obstacles to any study of acupuncture is a single standard of care," said Dr. Pak H. Chung of New York Weill Cornell Medical Center. "Only appropriate training and certification of acupuncture practitioners by state agencies can facilitate the integration of acupuncture into the treatment of female infertility, and health care in general."

The lead review article reports that acupuncture treatment has the following potential fertility-boosting benefits:

- ☐ Increased blood flow to the uterus and therefore uterine wall thickness, an important marker for fertility
- ☐ Increased endorphin production, which, in turn, has been shown to effect the release of a gonadotropin-releasing hormone (GnRH), a decapeptide involved in regulating reproduction
- ☐ Lower stress hormones responsible for infertility
- ☐ Impact on plasma levels of the fertility hormones: follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and Progesterone (P)
- ☐ Normalization of the hypothalamic-pituitary-ovarian axis, a key process in fertility
- ☐ A positive effect for women with polycystic ovarian syndrome, a hormonal imbalance present in three percent of adolescents and adults.

Acupuncture is the manipulation of thin metallic needles inserted into anatomically defined locations on the body to affect bodily function. These so-called acupoints correspond to areas on the surface of the body that have been shown to have greater electrical conductance due to the presence of a higher density of gap junctions along cell borders. A greater metabolic rate, temperature, and calcium ion concentration are also observed at these points.

Dr. Rosenwaks, Director of the Center for Reproductive Medicine and Infertility, and Dr. Chung treat infertility patients at Weill Cornell Medical Center at New York-Presbyterian Hospital. Dr. Rosenwaks is the Revlon Professor of Reproductive Medicine in Obstetrics and Gynecology, Co-Director of the Institute for Reproductive Medicine, and Attending Obstetrician and Gynecologist at Weill Cornell. Dr. Chung is Assistant Professor of Reproductive Medicine, Assistant Professor of Obstetrics and Gynecology, and Assistant Attending Obstetrician and Gynecologist at Weill Cornell. Dr. Chang is Clinical Assistant Professor of Medicine at Weill Cornell, where he teaches courses on alternative and complementary medicine. He is also affiliated with Meridian Medical.

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In this evaluation of nearly 200 infertile women, high levels of religiosity and spirituality are significantly correlated with low levels of psychological distress. Clinicians should be prepared to discuss religious and spiritual issues with their patients, as those issues may play an important role in the psychological health of infertile women—and in their response to infertility treatment.

Key Points

Women who experience infertility are more likely to suffer from heightened depression and anxiety symptoms than women in general. The longer the infertility and the greater the associated distress, the more likely a patient will suffer depressive symptoms, which can in turn diminish fertility and interfere with treatment success.

A high level of spiritual well-being is significantly linked with less infertility distress and fewer depressive symptoms, suggesting a relationship between spirituality and the psychological well-being of women undergoing infertility treatment. Conversely, self-reported depressive symptoms and lower levels of spiritual well-being predict higher levels of infertility distress.

Spirituality and religion are important sources of solace for most individuals. Many physicians do not describe themselves as personally religious, and most are not trained to discuss spiritual matters with patients. However, physicians may be well-advised to inquire about and support patients' religious beliefs to help promote their physical and psychological well-being.

Patients with strong religious faith, studies show, sustain medical crises with better outcomes than those who do not hold strong spiritual beliefs or maintain religious practices.[1] Research also shows that religious people tend to have a greater sense of well-being,

greater life satisfaction, lower levels of depression and anxiety, and a decreased risk of suicide.[2–7]

Society, especially religious society, has traditionally valued woman for her life-giving role. So it is not surprising that living with unrealized hopes for a child can negatively affect a woman's psychological well-being.

Infertility and depression

Women who experience infertility report significantly higher levels of depressive symptoms[8] and anxiety[9] than women in the fertile population. In one study, 11% of infertile subjects met the criteria for a major depressive episode, compared to 3.6% of fertile subjects.[10] In another study of infertile women, half of the subjects reported changes in their sexual function, and 75% reported changes in mood, such as increased feelings of sadness.[9]

There is also evidence that depressive symptoms are associated with decreased fertility and can interfere with the success of infertility treatment. In one investigation, women with a lifetime history of clinical depression were nearly twice as likely to report infertility as those not depressed.[11] *In vitro* fertilization (IVF) patients who reported heightened levels of depressive symptoms prior to beginning IVF treatment had significantly lower success rates than women with lower levels of depressive symptoms.[12,13] In general, infertility-related stress has been found to have direct and indirect adverse effects on treatment outcomes.[14]

In caring for the infertile woman, then, it is important to identify and minimize factors that lead to depressive symptoms and psychological distress and to identify and emphasize factors that are associated with healthy coping. If, for example, religious women were to report lower levels of depressive symptoms or psychological distress during infertility than less religious women, health care professionals might want to encourage their patients to consider religious or spiritual practices that have provided comfort in the past.

One study was located which considered the relationship between faith and fertility, and it only assessed Jewish women in Israel. Women undergoing IVF were asked to indicate their attitude toward religion and to classify themselves as secular, traditional, or orthodox.[15] The

study uncovered a significant relationship between religious observance and conception rates, with the highest rates in the traditional group. Of those who conceived, 70% classified themselves as "traditional." Among the women who didn't conceive, only 27% reported traditional religious practice. Folkman and Chesney suggest that "reliance on spiritual beliefs and engaging in spiritual activity have been identified as ways of coping in stressful situations."[16] Adherence to traditional faith may provide an effective way to cope with adversity and disappointment.

Religion's advantages— and possible drawbacks

Strong religious beliefs may help or interfere with coping and healing. On the one hand, some may find comfort by believing that infertility is part of a divine plan, while others may interpret infertility as punishment from a higher power for past sins and indiscretions. Some infertile women who display strong religious or spiritual beliefs may achieve relaxation through prayer. Others may experience heightened levels of distress from feeling that their prayers for a child have gone unanswered, or from agonizing over whether to pursue a treatment that may be specifically banned by their religion.

Being religious may benefit the infertility patient by providing a feeling of community and reducing social isolation. Conversely, a religious perspective may heighten a woman's sense of social isolation from a religious community that emphasizes childbearing. Infertile couples may be stigmatized by religious doctrines that make parenthood a core identity, such as the Old Testament commandment to "be fruitful and multiply" (Genesis 1:28). Thus, religious beliefs may actually compound the negative psychological effects of infertility.[17]

An investigation

In order to explore these connections in further detail, the authors conducted a study to investigate the role of religiosity and/or spirituality in shaping the subjective psychological well-being of infertile women. The 3 specific questions addressed in this research were:

(1) Is there a relationship between spirituality (either religious well-being or existential well-being) and depressive symptoms?

(2) Is there an association between increased spirituality and decreased infertility distress? and

(3) Do women who report more depressive symptoms also report more distress with their infertility?

Materials and methods

Subjects

Those eligible to participate included all English-speaking women who had a scheduled appointment or procedure at any of the 4 offices of Boston IVF, a hospital-affiliated infertility practice, during the recruitment phase (March 1 through August 1, 2000).

Materials

Each participant was asked to complete 4 questionnaires while waiting for an appointment with an infertility specialist. The questionnaires included:

- the Beck Depression Inventory,[18] which measures depression severity and is a widely-used self-report instrument for assessing depression

- the Fertility Problem Inventory,[19] which measures perceived infertility-related stress and identifies and measures domains of stress specific to infertility such as social, sexual, and relationship concerns

- the Spiritual Well-Being Scale,[20] evaluating the quality of one's spiritual health in a 2-dimensional construct: religious well-being and existential well-being, and

- a demographic form, collecting information on religious denomination, current religious practices, infertility history, and mental health history.

The Spiritual Well-Being Scale was developed to measure the quality of spiritual health. It has two constructs—religious well-being (well-

being as it relates to God) and existential well-being (well-being as it relates to life satisfaction).

Methods

The study was approved by the Institutional Review Board of the Beth Israel Deaconess Medical Center in Boston. Each questionnaire packet included a letter from the investigators explaining the study's purpose and procedures. It informed the prospective subject that completing the questionnaires implied consent to become a participant.

During the recruitment phase, each reception desk at the 4 Boston IVF waiting rooms had a sign indicating that any woman undergoing any infertility treatment was eligible. The sign also explained that participants would remain anonymous, that the study would take approximately 20 minutes to complete, and that participants would receive a free relaxation audiotape when they handed in the questionnaires.

Women who expressed interest received a packet that included the introductory letter from the investigators, the questionnaires, and a set of directions.

Statistical analysis

Participants were requested to fully complete all 4 questionnaires, though not all were expected to do so (actually, 168 of 195 participants completed all 4). At present, there are no formal procedures for scoring Beck Depression Inventory, Fertility Problem Inventory, and Spiritual Well-Being Scale questionnaires that are missing data.[17–19] Prior to beginning the study, we elected to use the standard scoring procedures of a widely used anxiety questionnaire (State-Trait Anxiety Inventory) for scoring and weighing questionnaires that were missing data.[21]

Separate linear regression models (stepwise algorithm) were used to determine if demographic, religious, or medical characteristics were independent predictors of depressive symptoms (Beck Depression Inventory) or infertility distress (Fertility Problem Inventory). Predefined potential predictors included a number of continuous variables (age, education, duration of infertility, duration of infertility

treatment, Spiritual Well-Being Scale, and, depending on the regression model, Beck Depression Inventory or Fertility Problem Inventory.) Potential predictors also included a number of discrete variables (marital status, employment status, number of children [if any], whether or not each subject considered herself to be optimistic or pessimistic, religious education, frequency of attendance at religious services, current religious practice, prayer or meditation, lifestyle change in religion, infertility diagnosis, and number of office visits.) Variables were included in the final Beck Depression Inventory model or Fertility Problem Inventory model if they had a *P* value of 0.05 or less. Statistical analyses were performed using SPSS 10.1 (www.spss.com).

Results

Participants included a total of 195 women who were currently seeking treatment at Boston IVF. Their ages ranged from 22 to 47 years (mean age, 36.4 years), and approximately 61% were in their 30s (Table 1). Half of the group had some level of graduate education, and a large majority of the women, 78%, were married. Approximately 86% were currently employed, and over three-quarters (77%) did not already have a child.

TABLE 1.

Demographic characteristics of the study population

	Participants (n = 195)
Mean age in years (± sd)	36.4 (5.2)
Age (n, %)	
<30	17 (8.7)
30–34	58 (29.8)
35–39	60 (30.7)
40–44	46 (23.6)
>45	13 (6.7)
Missing	1 (0.5)
Mean education in years (± sd)	16.8 (2.3)
Education (n, %)	
High school degree	12 (6.2)
Some college	25 (12.8)
College degree	59 (30.3)
Graduate work	96 (49.2)

	Participants (n = 195)
Missing	3 (1.5)
Marital status (n, %)	
Single	11 (5.6)
Committed relationship	25 (2.6)
Married	152 (77.9)
Divorced	1 (0.5)
Missing	26 (13.3)
Currently employed (n, %)	
Yes	167 (85.6)
No	28 (14.4)
Already have a child (n, %)	
Yes	43 (22.1)
No	150 (76.9)
Missing	2 (1.0)
Optimistic/pessimistic (n, %)	
Optimistic	48 (24.6)
Pessimistic	136 (69.7)
Missing	11 (5.6)

The religious demographics in Table 2 indicate that 75% of the subjects were Christian (52% Catholic), 17% were Jewish, 4% had "other" religions, and 2% reported no religious affiliation. The vast majority (85%) had received some formal religious education. Some 65% reported "regular" religious attendance. About 35% said they attended religious services at least monthly, while 30% said they never attended services. A sizeable majority of respondents, 79%, said they currently pray or meditate. Nearly one quarter of the subjects (24%) reported becoming more religious since experiencing infertility, while 4.6% reported a decline in religiosity.

TABLE 2.

Religious characteristics of the study population

	Participants (n = 195)
Religious denomination (n, %)	
Catholic	101 (51.8)
Protestant	35 (17.9)
Other Christian	10 (5.1)
Jewish	34 (17.4)
Other	8 (4.1)

	Participants (n = 195)
None	4 (2.1)
Missing	3 (1.5)
Religious education (n, %)	
Religious school	58 (29.7)
Occasional studies/Sunday school	108 (55.4)
None	15 (7.7)
Missing	14 (7.2)
Current religious service attendance (n, %)	
Yes	126 (64.6)
No	68 (34.9)
Missing	1 (0.5)
Frequency of religious service attendance (n, %)	
Daily	2 (1.0)
Weekly	51 (26.2)
Monthly	16 (8.2)
Religious holidays only	21 (15.4)
Several times a year	30 (10.8)
At least once a year	15 (7.7)
Never	59 (30.3)
Missing	1 (0.5)
Current religious practice (n, %)	
Secular	50 (25.6)
Traditional	74 (37.9)
Religious	48 (24.8)
Very religious	13 (6.7)
Missing	10 (5.1)
Currently pray or meditate (n, %)	154 (79.0)
Lifestyle change in religion (n, %)	
More active	46 (23.6)
Less active	9 (4.6)
No change	136 (69.7)
Missing	4 (2.1)

The mean duration of infertility among participants was 31 months, and the mean duration of infertility treatment was 18.4 months (Table 3). One quarter of the subjects had been attempting to conceive for more than 3 years, whereas over half (56%) reported infertility treatment of 1 year or less. Infertility diagnoses varied: 36% involved female factor infertility, 6% male factor, and 11% both female and male factor. More than a third (37%) had “unexplained” infertility.

TABLE 3.

Medical characteristics of the study population

	Participants (n = 195)
Mean duration of infertility in months (± sd)	31.0 (24.2)
Duration of infertility (n, %)	
0–12 months	22.5
13–24 months	33.1
25–36 months	17.4
37+ months	24.4
Missing	2.6
Mean duration of infertility treatment in months (± sd)	18.4 (20.4)
Duration of infertility treatment (n, %)	
0–6 months	31.3
7–12 months	25.1
13–24 months	22.6
25+ months	20.0
Missing	1.0
Infertility diagnosis (n, %)	
Female factor	71 (36.4)
Male factor	12 (6.2)
Male and female factor	22 (11.3)
Unexplained	72 (36.9)
No diagnosis	12 (6.2)
Missing	6 (3.1)
Number of office visits (n, %)	
First visit	16 (8.2)
1–4 visits	30 (15.4)
5–9 visits	31 (15.9)
10–14 visits	29 (14.9)
15 or more visits	88 (45.1)
Missing	1 (0.5)

The mean score (± SD) on the Beck Depression Inventory was 10.6 (7.4), with a range of 0 to 39. On the Beck scale, a score of 10 to 18 indicates mild to moderate depression, 19 to 29 reflects moderate to severe depression, and 30 or more describes severe depression. For the Fertility Problem Inventory, the mean score was 148.1 (32.8) in a range of 67 to 219. For the Spiritual Well-Being Scale, the mean was 88.6 (16.5) in a range of 47 to 120. A low score on the Spiritual Well-

Being Scale is 20 to 40, a medium score is 41 to 99, and a high score is 100 to 120.

To determine whether scores on the Beck and Fertility Problem instruments could be independently explained by demographic, religious, or medical factors, 2 separate stepwise linear regression models were conducted:

- Table 4A shows the final Beck model. Three factors—elevated fertility distress on the Fertility Problem Inventory, a greater number of fertility specialist office visits, and lower spiritual well-being on the Spiritual Well-Being survey—predicted higher levels of depressive symptoms on the Beck Depression Inventory.

TABLE 4A.

Multiple linear regression for Beck Depression Inventory model (n = 168)

Predictor	**Beta**	**t**	***P* value**
Fertility Problem Inventory	0.606	8.758	0.0005
Spiritual Well-Being Scale	−0.211	−3.056	0.003
Number of office visits	0.133	2.003	0.048

- Table 4B shows the final Fertility Problem Inventory model. Two factors—elevated depressive symptoms on the Beck scale and a longer duration of infertility—were predictive of increased fertility distress scores on the Fertility Problem Inventory.

TABLE 4B.

Multiple linear regression for Fertility Problem Inventory model (n = 168)

Predictor	**Beta**	**t**	***P* value**
Beck Depression Inventory	0.674	89.984	0.0005
Duration of infertility	0.186	2.752	0.007

There were significant positive correlations between the Beck Depression Inventory, Fertility Problem Inventory, duration of infertility, and number of office visits. Significant negative correlations were exhibited for the Spiritual Well-Being Scale and both the Beck Depression Inventory and Fertility Problem Inventory (Table 5).

TABLE 5.

Correlations

	Fertility Problem Inventory	Duration of infertility	Beck Depression Inventory	Number of office visits
Duration of infertility	.232*			
Beck Depression Inventory	.715**	.170*		
Number of office visits	.173*	.223**	.229**	
Spiritual Well-Being Scale	−.301**	.019	−.361**	−.124

Correlations for 168 subjects who completed all questionnaires.

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

Discussion

The survey results suggest a link between depressive symptoms and fertility distress in women undergoing infertility treatment. Additionally, there is an inverse correlation between spiritual well-being and depressive symptoms and fertility distress.

A majority of Americans report some degree of religious belief. Recent surveys indicate 94% of Americans believe in a god, 63% belong to a church, 44% have attended religious services within the past week, and 59% say religion is "very important" in their lives.[22] Spirituality and religion are thus important sources of solace and support for most individuals.

Health professionals, however, tend to be less religious than the general public. In one study, while 72% of the American public reported that "my whole approach to life is based upon my religion," only 39% of psychiatrists and 33% of psychologists felt the same way.[23] In a study comparing the spiritual views of family physicians

and their patients, patients were more likely to believe in God (91% vs 64%) and the afterlife (60% vs 45%). Patients were also more likely than their physicians to pray (85% vs 60%) and to feel close to God (74% versus 43%).[24]

Religious variables are often neglected in clinical research and medical practice. In a 1993 study of 1,666 abstracts published in *Clinical Research*, only 1% included religious measures.[25] In a study of hospitalized patients, 80% reported their physician rarely or never addressed spiritual issues, although 77% believed they should. Nearly half (48%) wanted their doctor to pray with them, and 42% wished their physician would ask them about their faith healing experiences.[26]

In a national poll of 1,000 adults, 63% wanted their physician to talk to them about their spiritual health, but only 10% reported their physician had done so.[27] In another poll of 1,004 adults, 82% of respondents reported belief in the healing power of prayer, and 64% stated physicians should pray with their patient, if requested to do so.[28]

Of 212 studies examining the impact of religious commitment on health, 75% demonstrated a positive effect, 17% demonstrated either a mixed effect or no effect, and 7% demonstrated a negative effect.[1] Many of these researchers believe the power of religious belief can promote healing in some patients. Religious belief can provide the positive psychological effect of social support, improve health habits associated with regular church attendance, or make stress-system changes that have important stabilizing effects on the nervous system and the hypothalamic-pituitary axis.[29]

The results of our study suggest that infertile women with higher levels of spiritual well-being as measured by the Spiritual Well-Being Scale also report fewer depressive symptoms and less overall distress from their infertility experience. Depressive symptoms and infertility-generated distress are unpleasant. Such distress may lead to termination of infertility treatment. Distressed patients may be less compliant and more difficult to work with. Depressive symptoms are also associated with lower pregnancy rates in infertility treatment.[8,12,13]

While the study results show a relationship between spiritual well-being and distress during infertility treatment, one should not conclude that high levels of spiritual well-being will diminish depressive

symptoms or infertility-associated distress. Depending on the patient and the circumstances, religious issues can be either healing or disruptive. Therefore, it may not be clear to physicians whether to encourage patients to discuss their religious beliefs and practices.[29]

Talking about religion with patients

Most physicians are not trained to converse with patients about their spiritual lives. To better understand the patient's concerns about infertility, however, it may be helpful to address spiritual and religious beliefs. It has been suggested that when a physician ignores these beliefs, the diagnostic assessment is incomplete.[30] Health care professionals may be well-positioned to appreciate and support the spiritual journeys of their patients.[31]

If physicians do choose to inquire about the spiritual and religious beliefs of their patients, they could use a simple open-ended question such as, "Do you have any religious or spiritual practices that you would like me to know about?"[32] A panel of the American College of Physicians suggests other questions, including:

- Is faith (religion, spirituality) important to you?
- Has faith been important to you at other times in your life?
- Do you have someone to talk to about religious matters?
- Would you like to explore religious matters with someone?[33]

Physicians and other health care professionals can also support religious beliefs that aid in coping, including rituals to mark events such as a miscarriage or stillbirth.[34] Physicians should not prescribe activities that impose their own religious views or initiate prayer unless they know the patient's religious background and the patient has specifically requested it. Physicians should not provide in-depth religious counseling, which is best handled by trained clergy.[30,33]

In summary, spirituality appears to play an important role in the psychological health of infertile women. Future studies are needed to replicate and expand these findings. Health care professionals may be well-advised to inquire about and support their patients' religious beliefs to promote the physical and psychological well-being of infertile women.

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A Randomized Study Evaluating Acupuncture as an Adjunct to IVF

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Background and Significance: Acupuncture is based on patterns of energy flow (Qi) through a network of meridians. Maintenance of Qi is essential for health, while blockage may provoke disease. Acupuncture works by unblocking the various types of stagnation that can occur along these channels. After Paulus et. al demonstrated the benefit of acupuncture to IVF (Fertil Steril Apr 2002), many IVF centers began offering these services.

Objective: The purpose of this study was to determine if there are benefits of standard acupuncture compared to sham acupuncture as an adjunct to IVF.

Materials and Methods: A randomized, controlled, double-blind, cross-over pilot trial was performed using a needle-like device (sham acupuncture) as a control. Approval from GAMC's Investigational Review Board was acquired. Inclusion criteria were women aged 18 to 42 years with a history of failed IVF cycle(s); day 3 FSH ≤20 IU/ml; the presence of both ovaries; and a normal uterine cavity. Exclusion criteria was Kruger morphology <4%.

Results: Seventeen subjects were enrolled and seven subjects completed both arms of the study. The mean age was 36.2 years (range 28-41 years). The mean Day 3 FSH=6.8 IU (range 3-13 IU). There were four ongoing pregnancies after the first cycle, equally distributed. Seven subjects were crossed over after the first cycle. Of these, four from the standard acupuncture group and one from the sham acupuncture group attained pregnancy. Two subjects of the standard acupuncture group were on-going pregnancies and one from the sham group. Only the sham group had two IVF cancellations. An unpaired Mann-Whitney Test using a two-sided p value was performed.

	Sham	**Standard**

	acupuncture	**acupuncture**
Oocytes retrieved	11 ±8.8	12 ±7.3
Mature oocytes	7.4 ± 5.5	8.7 ±5.2
Normal fertilization	5.9 ±3.9	7.5 ±6.0
Amount of gonadotropins (IU)	5085 ±1138	3400 ±1413*
Endometrial thickness (mm)	9.6 ±3.4	12.2 ±3.1
Embryos transferred	2.8 ± 1.97	2.6 ±1.31
Chemical pregnancies	3	7
Clinical pregnancies	3	5
Ongoing pregnancies	3	4

*statistically significant $p<0.05$

Note: results reported as mean ±SDwhere applicable

Conclusions:Our study shows a significantly lower amount of gonadotropins used when IVF is combined with standard acupuncture. A 70% pregnancy rate was also achieved with standard acupuncture and IVF, compared to 25%. Larger prospective trials are necessary.

Reduction of blood flow impedance in the uterine arteries of infertile women with electro-acupuncture

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Source: *European Society for Human Reproduction and Embryology*

In order to assess whether electro-acupuncture (EA) can reduce a high uterine artery blood flow inpedance, 10 infertile but otherwise healthy women with a pulsatility index (PI) ≥3.0 in the uterine arteries were treated with EA in a prospective, non-randomized study. Before inclusion in the study and throughout the entire study period, the women were down-regulated with a gonadotrophin-releasing hormone analogue (GnRHa) in order to exclude any fluctuating endogenous hormone effects on the PI. The baseline PI was measured when the serum oestradiol was ≤0.1 nmol/l, and thereafter the women were given EA eight times, twice a week for 4 weeks. The PI was measured again closely after the eighth EA treatment, and once more 10-14 days after the EA period. Skin temperature on the forehead (STFH) and in the lumbosacral area (STLS) was measured during the flrst, fifth and eighth EA treatments. Compared to the mean baseline PI, the mean PI was significantly reduced both shortly after the eighth EA treatment ($P < 0.0001$) and 10-14 days after the EA period ($P < 0.0001$). STFH increased significantly during the EA treatments. It is suggested that both ot these effects are due to a central inhibition of the sympathetic activity.

Key words: electro acupuncture/pulsatilily index (PI)/trans-vaginal colour Doppler curve/uterine artery blood flow

Introduction

Successful in-vitro fertilization (IVF) and embryo transfer demand optimal endometrial receptivity at the time of implantation. Blood flow

impedance in the uterine arteries, measured as the pulsatility index (PI) using transvaginal ultrasonography with pulsed Doppler curves, has been considered valuable in assessing endometrial receptivity (Goswamy and Steptoe, 1988; Sterzik *et al.*, 1989; Steer *et al.*, 1992, 1995a,b; Coulam *et al.*, 1995; Tekay *et al.*, 1995). Steer *et al.* (1992) found that a PI ≥3.0 at the time of embryo transfer could predict 35% of the failures to become pregnant. Coulam *et al.* (1995) did not observe any significant differences between PI measurements done on the day of oocyte retrieval compared with PI measurements on the day of embryo transfer. This would allow prediction of non-receptive endometria earlier in the cycle.

Previous studies on rats have shown a decreased blood pressure after electro-acupuncture (EA) with low frequency (2 Hz) stimulation of muscle afferents (A-d fibres). The decreased blood pressure was related to reduced sympathetic activity (Yao *et al.*, 1982; Hoffman and Thoren, 1986; Hoffman *et al.*. 1987, 1990a,b), and was paralleled by an increase in the ß-endorphin concentration in the cerebrospinal fluid (CSF), suggesting a causal relationship to central sympathetic inhibition (Cao *et al.*, 1983; Moriyama 1987; Reid and Rubin, 1987). The cardiovascular effects of acupuncture treatment are probably mediated by central opioid activity via the ß-endorphin system from the hypothalamus.

The aim of this study was to evaluate whether EA can reduce a high impedance in the uterine arteries. There are several conceivable mechanisms which may give this effect.

In addition to central sympathetic inhibition via the endorphin system, vasodilatation may be caused by stimulation of sensory nerve fibres which inhibit the sympathetic outflow at the spinal level, or by antidromic nerve impulses which release substance-P and calcitonin gene-related peptide from peripheral nerve terminals (Jansen *et al.*, 1989; Andersson, 1993; Andersson and Lundeberg, 1995).

It has been assumed that various disorders in the autonomic nervous system, such as hormonal disturbances, may be normalized during auricular acupuncture (Gerhard and Postneck, 1992). It has also been suggested that the concentrations of central opioids may regulate the function of the hypothalamic-pituitary-ovarian axis via the central sympathetic system, and that a hyperactive sympathetic system in anovulatory patients could be normalized by EA (Chen and Yin, 1991).

Materials and Methods

Subjects, design and PI measurements

The study was approved by the ethics committee of the University of Gothenburg and was conducted at the Fertility Centre Scandinavia, Gothenburg, Sweden, a tertiary private IVF unit. All women attending the clinic for information about the IVF/embryo transfer procedure, had the PI of their uterine arteries measured by transvaginal ultrasonography and pulsed Doppler curves (Aloka SSD 680: Berner Medecinteknik, Stockholm, Sweden). The PI value for each artery was calculated electronically from a smooth curve fitted to the average waveform over three cardiac cycles, according to the formula: PI = (A - B)/mean, where A is the peak systolic Doppler shift, B is the end diastolic shift frequency and mean is the mean maximum Doppler shifted frequency over the cardiac cycle. A reduction in the value of PI is thought to indicate a reduction in impedance distal to the point of sampling (Steer *et al.*, 1990).

In the routine preparation for their IVF/embryo transfer treatment, all women were down-regulated with a gonadotrophin-releasing hormone analogue (GnRHa) (Suprecur: Hoechst. Germany). When their oestradiol concentration in serum was <0.1 nmol/1, the women were considered down-regulated and the PI of their uterine arteries was again measured in those women showing a mean PI ≥3.0 before down-regulation. The measurements were done by two of the authors (M.W. and U.W.) between 08.30 h and 14.30 h. These hours were chosen for practical reasons, and also to reduce the risk that the PI measurements would be affected by the circadian rhythm in blood flow, recently reported by Zaidi *et al.* (1995). Three measurements were made on the right and three on the left uterine artery of each patient. Before the study was conducted, the observers were well trained in PI measurements with the equipment used. Steer *et al.* (1995) has shown that in trained hands, the inter-, and intra-observer variations in vaginal colour Doppler ultrasound are sufficiently small to provide a basis for clinically reliable work.

PI measurements were done on all women attending the unit for an IVF/embryo transfer treatment between November 1992 and February 1993. Of these, all infertile but otherwise healthy women, with a mean PI ≥3.0 in the uterine arteries both before and after down-regulation, were invited to be included in the study.

In all, 10 women accepted after informed consent and they had a mean age of 32.3 years (range 25-40 years). The infertility diagnoses were unexplained infertility (n = 6), tubal factor (n = 3) and polycystic

ovarian syndrome (*n* = 1).

From their inclusion and onwards, the women were kept on the GnRHa and were given no other pharmacological treatment. Consequently, their gonadotrophins and ovarian steroids were kept at a constantly low concentration, both at their inclusion in the study and throughout the whole study period. Thus, PI changes due to hormonal fluctuations were avoided.

EA was then given eight times, twice a week for 4 weeks. The mean PI of the uterine arteries was measured (mean of three PI on each side) directly after the eighth EA treatment and again 10-14 days after the EA period.

Of the 10 women included, two were later excluded. One of them, with tubal factor infertility, was excluded because she started taking medications for her migraine, which could have affected her PI. The other excluded woman, with unexplained infertility, stopped her GnRHa treatment because she preferred IVF/embryo transfer in a natural cycle.

Acupuncture Treatment

The sympathetic outflow may be inhibited at the segmental level and, for this reason, acupuncture points were selected in somatic segments according to the innervation of the uterus (Thl2-L2, S2-S3) (Bonica, 1990).

The needles were inserted i.m. to a depth of 10-20 mm. The aim of the stimulation was to activate group III muscle-nerve afferents. The needles were twirled to evoke `needle sensation,' often described as tension, numbness, tingling and soreness, sometimes radiating from the point of insertion. The needles were then attached to an electrical stimulator (WQ-6F: Wilkris & Co. AB, Stockholm, Sweden) for 30 min. The location of the needles was the same in all women (Table I).

Table 1. Acupuncture points, their anatomical position and their innervation

Points*	Segmental innervation (afferent muscle)	Muscle localization

BL 23	L1, 2, 3	Erector spinae thoracolumbale
BL 28	L4, 5, S1, 2, 3	Erector spinae lumbosacrale
SP 6	L4, 5, S2, 3	Tibialis posterior at the medial side
BL 57	S1, 2	Gastrocnemius and m. soleus at the dorsal side

*All were placed bilaterally.
BL - bladder channel.
SP - spleen channel.

Four needles were located bilaterally at the thoracolumbar and lumbosacral levels of the erector spinae, and were stimulated with high frequency (100 Hz) pulses of 0.5 ms duration. The intensity was low, giving non-painful paraesthesia.

Four needles were located bilaterally in the calf muscles, and were stimulated with low frequency (2 Hz) pulses of 0.5 ms duration. The intensity was sufficient to cause local muscle contractions.

Skin temperature

The skin temperature was measured with a digital infrared thermometer (Microscanner D-series: Exergen, Watertown, MA, USA) between the applied acupuncture needles in the lumbosacral region (25 mm from each needle), skin temperature lumbosacral (STLS), and on the forehead, skin temperature forehead (STFH). The measurements were made during the first, fifth and eighth EA treatments. The first measurements were made after 10 min rest, and just before the EA, these being considered as `baseline.' Thereafter, further measurements of STLS and STFH were done every seventh minute during the EA and immediately after the EA. The room temperature was constant during the three EA treatments.

Statistics

Analysis of variance (ANOVA: Newman-Keul's range test) was used to analyze the data.

Results

Blood flow impedance

Compared to the mean baseline PI, the mean PI was significantly reduced both soon after the eighth EA treatment (P < 0.0001) and 10-14 days after the EA period (P < 0.0001) (Figure 1), at which time six women had a mean PI <2.6 (Table II and Figure 2).

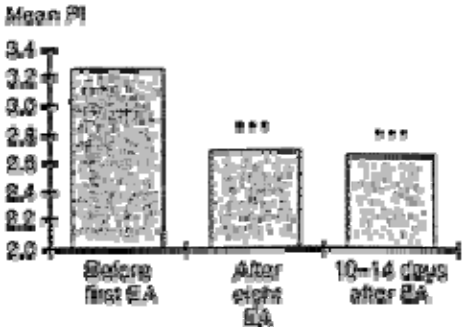

Figure 1. The mean pulsatility index (PI) (*n* = 8) for all women before the first electro-acupuncture (EA) treatment, immediately after the eighth EA treatment and 10-14 days after the EA period.
*** = significant changes (*P* < 0.0001) compared to the mean PI before the first EA treatment.

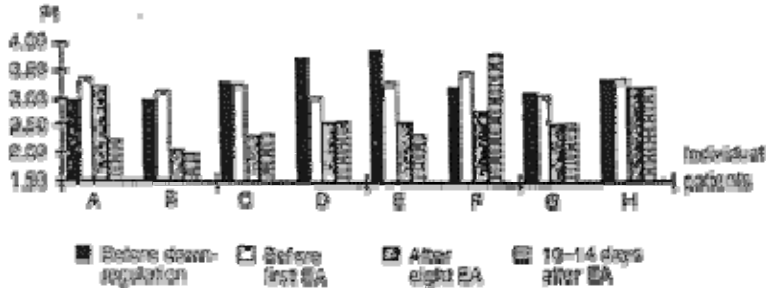

Figure 2. The individual mean pulsatility index (PI) before down-regulation, before the first electro-acupuncture (EA) treatment, immediately after the eighth EA treatment and 10-14 days after the EA period.

Table II. The individual mean pulsalility index (PI) before down-regulation, before the first electro-acupunclure (EA) treatment, immedialely after the eighth EA trealment, 10-11 days after the EA period, and average mean values

	Individual patients								
Pl value	A	B	C	D	E	F	G	H	Mean Value

Before down-regulation	3.00	3.00	3.30	3.75	3.90	3.25	3.14	3.33	3.34
Before EA	3.38	3.15	3.27	3.04	3.30	3.50	3.10	3.34	3.26
After eight EA	3.24	2.07	2.37	2.57	2.59	2.80	2.54	3.34	2.68
10-14 days after eight EA	2.25	2.01	2.40	2.60	2.40	3.84	2.54	3.20	2.65

The right and left uterine arteries responded similarly to EA. The diffcrence in mean PI between the two arteries was ≤0.3 (not significant), both before down-regulation, during down-regulation and throughout the whole study period. There was no significant difference in the mean PI for patients with different causes of infertility.

Skin temperature

The pooled results from all skin temperature measurements are presented in Figure 3. Compared with the starting point, mean STFH increased significantly after 21 min of EA (P = 0.02), and directly after the EA treatments (P = 0.002). STLS did not change significantly.

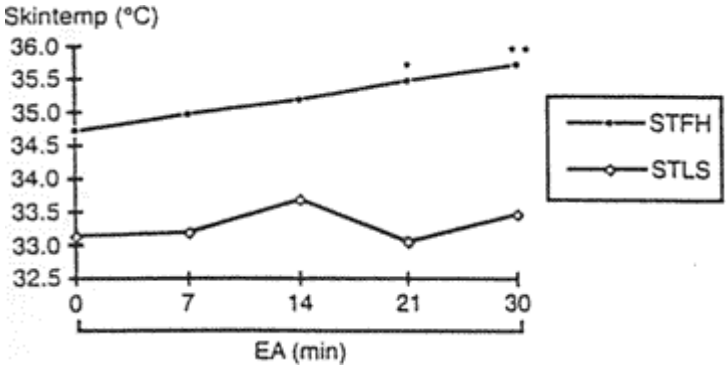

Figure 3. Pooled mean values (n = 8) of skin temperature on forehead (STFH) and skin temperature in the lumbosacral area (STLS) during the first, fifth and eighth electro-acupuncture (EA) treatments. * = significant changes (P = 0.02) after 21 min and ** = significant changes (P = 0.002) immediately after EA compared to the time just before needles were inserted. 0 = `baseline'.

Discussion

It has been shown in previous studies that a high PI in the uterine arteries is associated with a decreased pregnancy rate following IVF-embryo transfer (Goswamy *et al.*, 1988; Sterzik *et al.*, 1989; Steer *et al.*, 1992, 1995a.b; Coulam *et al.*, 1995). The results reported by

Tekay *et al.* (1995) support the hypothesis postulated by Steer *et al.* (1992) that uterine receptivity is improved when the PI value is between 2.0 and 2.99 on the day of embryo transfer. When a high PI is found before embryo transfer in a stimulated cycle, treatment options are few. Goswamy *et al.* (1988) successfully tried pre-treatment with exogenous oestrogens in the next cycle, but their results have not been verified by others. It has been proposed that the embryos should be frozen, thawed and transferred in an unstimulated cycle (Goswamy *et al.*, 1988; Steer *et al.*, 1992, 1994), but there is little support for the hypothesis that the PI would be lower under these contitions.

In experiments on spontaneously hypertensive rats, EA at low frequency (2-3 Hz) induced a long-lasting, significant fall in blood pressure which was associated with decreased activity in sympathetic fibres (Yao *et al.*, 1982; Hoffman and Thoren, 1986; Hoffman *et al.*, 1987, 1990a,b). A decrease in sympathetic activity appears to be generalized. In microneurographic studies on humans, EA in the upper limbs resulted in an initial increase and then a decrease in activity of sympathetic efferents in the tibial nerve, with a parallel increase in the temperature of the skin (Moriyama, 1987). Kaada (1982) reported that transcutaneous stimulation of acupuncture points in the hand increased the skin temperature, giving pain relief in limbs suffering from Reynaud's phenomenon. Kaada (1982) also found that electrical stimulation of accupuncture hand points in patients with ischaemic conditions of the lower limbs, increased the skin temperature in the lower limbs and possibly enhanced the healing of long-standing ulcers. It has been noted in both animals and humms that EA has greater effects on pathological conditions, e.g. hypertension or hypotension, whereas normal blood pressure is only slightly changed (Yao *et al.*, 1982: Hoffman and Thoren, 1986: Hoffman *et al.*, 1987, 1990a,b).

The mechanisms of sympathetic inhibition following EA are poorly understood. Based on animal experiments, Hoffmann and Thoren (1986) and Hoffman *et al.* (1987, 1990a,b) suggested that electrical slimulation of muscle efferents innervating ergoreceptors increases the eoncentration of ß-endorphin in the CSF. They found support for the hypothesis that the hypothalamic ß-endorphinergic system has inhibitory effects on the vasomotor centre, and thereby a central inhibition of sympathetic activity. It has been suggested that this central mechanism, involving hypothalamic and brain stem systems, is important in changing the descending control of many different organ systems, including the vasomotor system (Andersson. 1993; Andersson and Lundeberg, 1995).

In this study, the PI of the uterine arteries was signifieantly decreased soon after the eighth EA treatment and remained significantly decreased 10-14 days after the EA period. These findings suggest that a series of EA treatments increases the uterine artery blood flow. Another effect observed in this study was the signifieantly inereased STFH during the EA treatments.

The most likely cause of these effects is a decreased tonic activity in the sympathetic vasoconstrictor fibres to the uterus and an involvement of the central mechanisms with general inhibition of the sympathetic outflow, in accordance with previously observed EA effects (Kaada. 1982; Yao *et al.*, 1982; Cao *et al.*, 1983: Hoffman and Thoren, 1986; Hoffman *et al.*, 1987, 1990a,b; Moriyama, 1987; Reid and Rubin, 1987; Jansen *et al.*, 1989).

In conclusion. the present study showed a decrease of the PI in the uterine arteries following EA treatment. Randomized studies on a greater number of patients are needed to verify these results and to exclude non-specific effects.

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Acupuncture Normalizes Dysfunction of Hypothalamic-Pituitary-Ovarian Axis

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ABSTRACT

This article summarizes the studies of the mechanism of electroacupuncture (EA) in the regulation of the abnormal function of hypothalamic pituitary-ovarian axis (HPOA) in our laboratory. Clinical observation showed that EA with the effective acupoints could cure some anovulatory patients in a highly effective rate and the experimental results suggested that EA might regulate the dysfunction of HPOA in several ways, which rneans EA could influence some gene expression of brain, thereby, normalizing secretion of some hormones, such as GnRH, LH and E2. The effects of EA might possess a relative specificity on acupoints.

KEY WORDS: Electroacupuncture, ß-Endorphin, GnRH, LH, Estradiol, Estrogen receptor, Ovariectomized rat, Hypothalamic-pituitary-ovarian axis

INTRODUCTON

Acupuncture is a treasure of Chinese traditional medicine, which is employed in the treatment of different diseases, especially in relief of all kinds of pain [1, 2] over the world. Since 1960s we have used acupuncture with appropriate electro-stimulation to cure patients with anovulation disorder (sterility), the rate of EA induction of ovulation was increased from 50% initially to 80% presently. Other authors in China also reported that acupuncture was successfully to treat patients with sterility [3] and the lying-in woman with subnormal contraction of uterus [4]. All the above research demonstrates that acupuncture may be an effective curative method of some woman's diseases. However, many questions, such as "why", "how to" and "which" about the mechanism of EA effect are unknown. To address these problems we supposed that EA might influence the production and secretion of hormones, neurotransmitters or neuro-modulators of HPOA leading to the normalization of hormone status. We also noticed certain artides

reported that EA might affect the blood levels of LH, FSH, estradiol (E2) and prolactin in the female patients [4, 5, 6] and EA may be related to long term changes in gene expression [7, 8]. These results are all significant, yet insufficient to explain the mechanism of EA in the regulation of the function of HPOA. To obtain more data, a series of experimental studies in human and animal models has been performed in our laboratory.

MATERIALS AND METHODS

Selection and treatment of cases

Ten cases of chronically anovulatatory patients including eight cases of polycystic ovarian disease (POCA), one case of hypogonadotropic amenorrhoea and one case of oligomenorrhea were treated with EA in 13 menstruation cycles. They were all of productive age and the courses of disease were 3 to 12 years. On the 10th day of each menstruation cycle, the patients accepted the EA treatment. "Guanyuan(RN4)," "Zhongji(RN3)," "Sanyinjiao(SP6)," and bilateral "Zigong(EXCA1)" points were stimulated for 30 min at 8:00 AM, Q.D. for 3 days. The stimulation parameters were 7-8mA and 4-5 Hz with G6805 model generator. The electric current of EA was bearable well for every patient. The blood samples were collected from forearm of the patients one time per 15 min for detection of FSH.LH and ß-endorphin (ß-E).

Five health volunteers of a productive age with normal menstruation cycle were selected as controls, which were undergone the same treatment as above mentioned.

Animals and treatments

Wistar female rats weighting 200-250g were used. The half of animals were undergone ovariectomy and fed in the same environment with the intact rats at least for 15 days and vaginal smears were examined per day for 3 times. No exfoliative epithelium cell was found in the smears as an index for successfill ovariectomy. The ovariectomized rats and intact rats were randomly divided into two groups respectively: ovariectomized rat group (OVX), ovariectomized rat accepted EA treatment group (OVX+EA), intact rat group (INT) and intact rat accepted EA treatment group (INT+EA). The animals in OVX+EA and INT+EA received EA at the experimental acupoints of Guanyuan (RN4), Zhongji (RN3), Sanyinjiao (SP6) and bilateral Zigong (EXCA1) by EA apparatus (Model G6805-2, SMIF, Shanghai, China) with the frequency of 3 Hz and an intensity to produce a slight twitch of the limbs. After 3 days' treatment animals were given EA at

Waiguan (SJ5) and Huatuojiaji (EXTRA21) as the control acupoints in the same way (Fig 1). By the end of last experiment, animals were sacrificed and their adrenals, brains and pituitaries were taken out for detection of nucleolar oganizer regions (AgNORs) and hormones.

Pushpull perfusion in hypothalamic preoptic area (POA) and elution of pituitary and LH and ß-endorphin (ß-EP)

The technique of brain pushpull perfusion was processed as previously described by our laboratory [1]. The perfusate from hypothalamic POA was kept at -70°C for GnRX and ß-EP RIA.

The pituitaries were retrieved and put into 4°C cooled saline. Afterward, each pituitary was homogenized with 500µl of 70% acetone aqueous solution at 4°C. The homogenate was centrifugalized (2,000xg for 15 min at 4°C) and the supernatant was freeze-dried for LH and ß-EP RIA.

Radioimmunoassay (RIA) of hormones

GnRH IRA: GnRH content in the perfusate from rat hypothalamus was determined by RIA method developed by Nett in 1973 [9]. GnRH was iodinated by the modified chlomine-T technique[10]. Na125 I was manufactured by Radiochemical Center, Amersham.

ß-EP RIA: The sensitive radioimmunoassay was a routine in our laboratory [1]. The standards of human and rat ß-EP was synthesized by Peninsula Laboratories, Inc. and the rabbit antiserum of both ß-EP was developed in our laboratory. The cross-reaction from human ß-EP and camel ß-EP was detected about 20%. The sensitivity of this method was 10pg/tube.

LH, E2 and corticosterone RIA: LH, E2 and corticosterone RIA kits were bought from Shanghai Institute of Biologic Products, the Ministry of Health, P.R. China. All procedures of RIA were performed as described in the kit manuals.

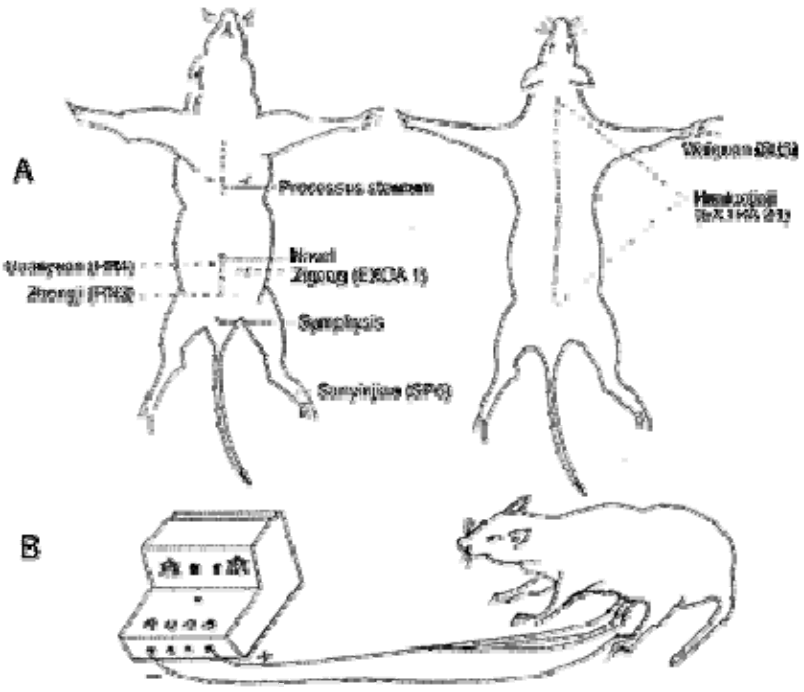

Fig. 1 A: Sketch of ventral view (left) and dorsal view (right) of rat shows the acupoints we used

B: Diagram shows the electroacupuncture procedures in conscious rat

Staining techniques: Vaginal smears were fixed by 100% ethyl alcohol, then stained with HE method. Adrenal sections were cut in 4μm thickness from paraffin blocks and processed with silver nitrate staining technique[11]. In each case, one hundred cells in zona fascicula were examined randomly under 100-fold oil immersion lens. Numbers and sizes of AgNOR dots were counted and measured.

C-fos protein immunohistochemistry: The inmunohistochemical analysis of c-fos expression in rat brain was perforrned as previously described[11].

Estrogen receptor (ER) protein immunohistochemistry (ABC method): Under sodium pentobarbital anesthesia (50 mg/kg, ip), the animals were perfused via left cardiac ventricle with 100ml of phosphate-buffered saline (PBS), followed by 300ml ice-cold fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH7.4). Afterwards, brain was removed with the same fixative for one day and immersed in 0. lM phosphate buffer containing 30% sucrose for another day. The hypothalamus blocks were frozen with dry ice and cut into 35 μM thick

section by cryostat. The brain sections were washed with 0.01M PBS for 15min x 3 and incubated in 0.01M PBS containing 0.5% Triton 100 and 3% normal goat serum (NGS) at 37°C-for one hour. Afterwards, the sections incubated in 1:1,000 ER monoclonal antibody (H222, Abott Co.) at 37°C for one hour, then at 4°C for two days. The sections, washed in PBS three times, were processed by ABC kit (from Vecot Labs) induding sequential incubation at 20°C in the following solutions with washes between them. (1). second antibody (dilution 1:100), 30min. (2). A+B reagents (dilutionl:100), 60min. (3). 0.05% diaminobenzidine/ 0.02% hydrogen peroxide in 0.1M Tris- HCI buffer (pH 7.2) 10min. The sections were washed in tap water, mounted and examined under light microscope. The certain areas of typical immunoreactive positive neurons were measured by computer image analysis system (Vecta PC).

ER mRNA hybridization: The total mRNA of brain was eluted by the modified phenol method [12]. ER cDNA probe (244bp) was labeled by the DlG-labeling kit (from Bohringman Co., Germany). The dot blot hybridization was processed as the method described by Sambrook J and his colleagues [13]. The dot blot images were analyzed with gray density by computer imaging analysis software (TJTY-300, from Tong - Ji university, Shanghai, China).

Statistics: All data in this paper were treated with analysis of variation (ANOVA), least significant difference (ISD) or student T-test.

RESULTS

Effect of EA on ovulatary induction and curing sterility in woman

After EA the blood ß-EP level of the patients resulting in ovulation either declined or maintain at the levels within the range of the normal levels and the ß-EP levels of those failing to show ovulation were significantly higher than the normal's' (table 1). On the other hand, the blood LH and FSH levels of the patients with ovulation after EA treatment tended to be the normal [14].

Table 1. Change of blood ß-EP level before and after EA (pg/ml)

Group of cases	N	Before EA	After EA
Ovulation	6	65.59 ± 24.15	*38.86 ± 10.11
No ovulation	7	65.59 ± 24.15	80.09 ± 22.16

Control	5	38.84 ± 10.13	41.52 ± 6.40

The values in this table are mean±SE, *P<0.05

Effect of EA on dysfunction of HPOA in ovariectomized rats

For a further study of the mechanism of EA effect on HPOA a series of experiments in the animal models was performed.

(1). EA induces maturation and exfoliation of vaginal epithelium cell and enhances blood level of E2.

After ovariectomy two weeks late, the exfoliated epithelium cell disappeared from the vaginal smears of the rats, but it reappeared in the smears following EA treatment. The blood level of E2 in OVX was increased significantly (table 2). No obvious change was seen in INT after EA treatment and in OVX following EA treatment with the control acupoints.

Table 2. The level of blood E2 following EA treatment (pg/ml)

Group	N	Before EA	After EA
OVX	10	*5.47 ± 0.63	**11.58 ± 0.98
INT	10	18.00 ± 3.26	18.34 ± 8.77

*P < 0.05 compared with INT, **P<0.01 compared with before EA

(2). EA promotes enlargement of adrenals and enhances activity of adrenal AgNORs as well as blood level of corticosterone

We found the adrenals of OVX+EA were enlarged and the weight of the adrenals was raised significantly. Using histochemical method, the AgNORs of the cells in inner adrenal cortex were examined. The result shows that the activity of AgNORs of OVX was enhanced (table 3, 4), and the level of blood corticosterone in OVX+EA was also increased (table 5). There were no similar effects in INT following EA treatment and in OVX after EA with control acupoints.

Table 3. AgNORs number in OVX and INT

Group	INT	INI+EA	OVX	OVX+EA	F value
N	4	3	4	7	
Number	1.55	1.19	1.25	2.53	

of AgNORs	1.82	1.28	1.61	2.05	
(mean/100 cells)	1.24	1.16	1.66	1.82	9.614*
	1.30		1.96	2.86	
				2.86	
				2.93	
				3.92	

*P < 0.01 tested with ANOVA

Table 4. Weight of adrenal

Group	INT	INI+EA	OVX	OVX+EA	F value
N	5	3	5	8	
Weight	57	54	45	67	
(mg)	56	57	68	72	
	57	58	56	66	5.825*
	43		50	71	
	57		58	57	
				74	
				74	
				68	

*P < 0.01 tested with ANOVA

Table 5. The levels of blood corticosterone in OVX and lNT (mean ± SE, ng/ml)

Group	N	Before EA	After EA
OVX	12	4.78 ± 0.42	*6.06 ± 0.73
INT	12	3.64 ± 0.15	4.76 ± 1.25

*P < 0.001 compared with before EA

(3). EA decreases the level of hypothalamic GnRH, pituitary LH and increases the contents of hypothalamic and pituitary ß-endorphin

After EA treatment the levels of GnRH released from hypothalamus was rnarkedly decreased however, the ß-endorphin (ß-EP) secretion in hypothalamus was raised. The pituitary content of LH was also fallen, but the ß-EP of pituitary was increased, as well as peripheral LH and ß-EP level (Fig.2).

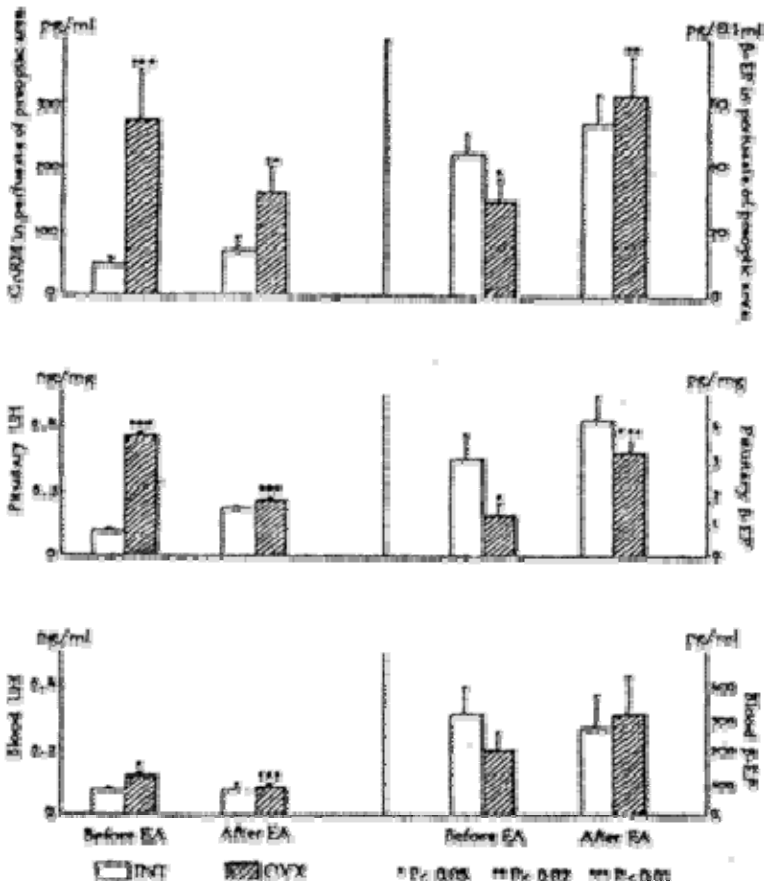

Fig. 2 Change of hypothalarnic GnRH and ß-EP, pituitary LH and ß-EP, blood LH and ß-EP before and after EA

Effect of EA on brain c-fos expression in ovariectomized rats

The area occupied by FOS protein labeled neuron was detected in medial preoptic nucleus (MPN), lateral preoptic nucleus (LPN), suprachiasmatic nucleus (SCN), paraventricular nucleus of the hypothalamus (PAVN), medial amygdala nucleus (MAN), periventricular nucleus of the hypothaLsmus (PVN), ventromedial nucleus of the hypothalamus (VNH) and arcuate nucleus (AR) 4 hours after ovariectomy (fig. 3a). The C-fos immunoreactive labeled neurons disappeared two weeks later following ovariectomy. The rats recovering for more than two weeks after ovariectomy, were received EA treatment. Many specific FOS labeled cells were observed in LPN, VNH, SCN and especially in POA, ARN, and PVN, but not any labeled neuron could be found in MAN. No obvious C-fos expression was shown in those nuclei in INT and INT+EA (fig. 3b).

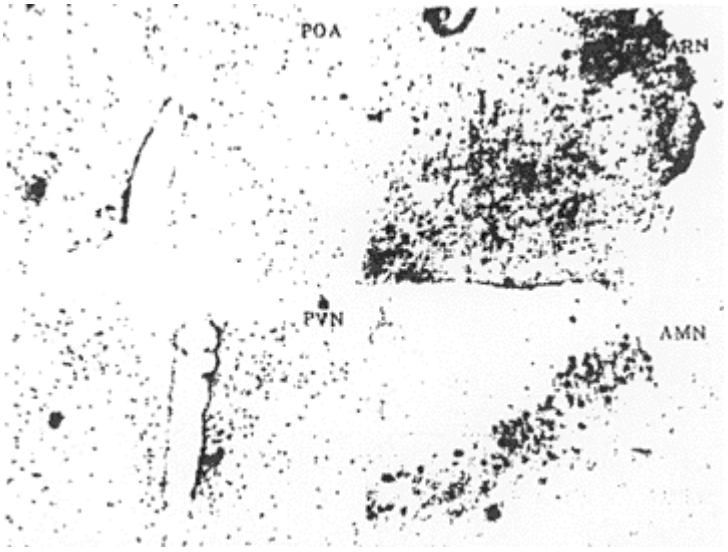

Fig. 3a C-fos immunocytochemistry neurons distribution after ovariectomy

Fig. 3b C-fos expression labeled neurons following electroacupuncture

<u>Effect of EA on expression of ER protein and ER mRNA in rat brain</u>
Estrogen receptor (ER) immunoreactive neurons were observed widely in rat brain with immunohistochemical technique, especially in MPN, ARN and VNH. The above nuclei were measured by computer image analysis system, and the results show that the mean gray density in OVX+EA was decreased apparently compared with that in OVX. Whereas there were no obvious changes of gray density levels in INT and INT+EA (fig, 4).

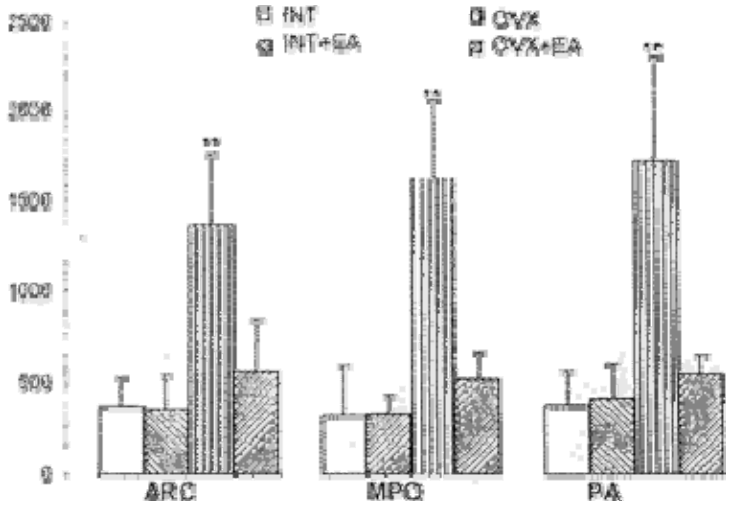

Fig. 4 Effect of EA on expression of ER protein in rat brain (Immunohistochernistry of monoclonal antibody) *p < 0.01 compared with OVX

The dot blot indicated that ER mRNA expression was increased about 48.11% in OVX compared with INT. The gray density of OVX was 129.75 ± l2.l3 and that in OVX+EA was 199.25 ± 5.75 attenuated significantly (Fig. 5). The gray density level in INT was 87.60 ± 5.91, and the level in INT+EA was 83.60 ± 4.83. There was no significant difference between INT and INT+EA

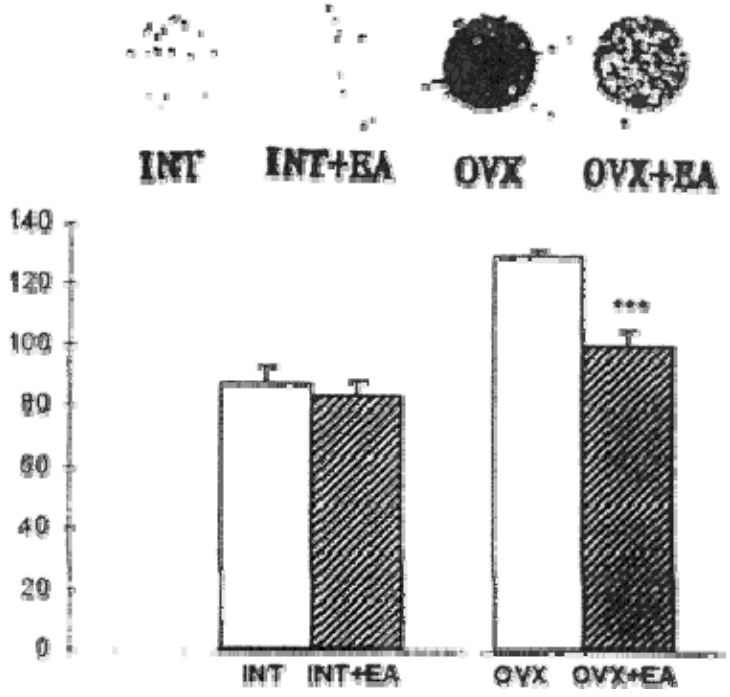

Fig. Effect of EA on expression of ER mRNA in rat brain (dot blot) ***

5 $p < 0.01$ compared with OVX

DISCUSSION

Since 1985 we have observed that the effect of EA ovulatary induction might relate to the hand skin temperature (HST) and the blood level of ß-EP [14]. On the other hand, after EA the blood FSH and LH levels of the patients who successfully ovulated either declined or maintained at normal. In general, provided that body temperature was normal and the environmental temperature was constant round 25°C, the HST may reflect the state of sympathetic system of a patient. These results suggest that in anovulatary cases the hyperactive sympathetic system can be depressed by EA and the function of HPOA can be regulated by EA through central sympathetic system. Moreover, EA may mediate the abnormal function via the influence on the secretion of the hormones in the different Level of HPOA.

To gain more evidences, we designed some animal experiments to explain the mechanism of EA effects on HPOA at the whole, cellular and molecular levels. We found that EA can induce maturation and exfoliation of vaginal epithelium cell in OVX rat. It is known that maturation and exfoliation of vaginal epithelium cells are a reaction dependent on estrogen level. So we determined the level of blood E2 in OVX and OVX+EA. The result shows the level of blood E2 in OVX was lower than that in normal, but it was increased significantly after OVX accepted EA treatment with the experimental acupoints. This result suggests EA might promote the activity of the compensative mechanism to elevate the subnormal level of E2 induced by ovariectomy in rats.

What is this compensative mechanism? To resolve this question, we considered that adrenal is the main organ to secrete sexual hormones except ovarian in females and observed the adrenals of the animals in four groups. The results show that the mean weight of the adrenal in OVX+EA was higher than that in OVX, INT and INT+EA, suggesting the adrenal function might be activated by EA. Subsequently, we detected that the number of AgNORs in zona fasciculata of OVX+EA was significantly increased. Nucleolar organizer regions (NORs) are loops of DNA, which possess ribosomal RNA (rRNA) genes. They are of vital significance in the ultimate synthesis of protein. Thus, the number and configuration of AgNORs (NORs stained by silver staining method) may reflect the activity of cell differentiation and transcription of nucleolar rDNA [15]. In the same time we found the content of blood corticosterone in OVX+EA was raised markedly, but there was no

change of blood corticosterone in OVX, INT and INT+EA. This result provided a further evidence that the adrenal cortex cells were initiated in OVX+EA.

The results including the changes of GnRH releasing from hypothalamus and of the pituitary and blood LH contents suggest that the effects of acupuncture in the regulation of HPOA may be exerted via to promote the function of hypothalamic pituitary-adrenal axis (HPAA), increasing the synthesis and secretion of adrenal steroid horrnones, the androgen of which then be transformed into estrogen in other tissues and thereby reset the negative feedback of estrogen to HPOA. Moreover, EA may accelerate the release of brain and pituitary ß-EP to inhibit the overnormal secretion of GnRH and LH that may be normalized.

Recently immunohistochemical analysis of the expression of oncogene c-fos ABl was induced by variety of stimuli [16, 17]. This represents a new method for mapping neuronal activity at the cellular level [18] and thus functionally and systematically tracing neuronal pathway in the nervous system (C NS) [19]. We used this method to examine the distribution of FOS labeled neuron in CNS for recovery of more evidences that EA may alter the neuroendocrine function of HPOA in ovariectomized rats in cellular and gene level. The results show that the specific FOS labeled neurons were observed especially in POA, ARN and PVN in OVX following EA treatment. In above nuclei there were a high concentration of GnRH and ß-EP neuron [20]. These results suggest this fact that the expression of FOS labeled neurons reappeared in above mentioned areas following EA treatment in ovariectomized rats may be related to the changes of GnRH and ß-EP from rat hypothalamus after EA treatment.

The level of estrogen in the body may regulate the expression of ER, which may by down-regulated following increase of estrogen level and up-regulated after decrease of estrogen [22]. Our finding that after decline of blood E2 induced by ovariectomy the expression of ER was increased and the expression of ER was inhibited by EA inducing the elevation of blood E2 are in accordance with these reported results. ER existing in the brain, especially in POA, ARN and VHN may mediate the function of neuroendocrine system [22, 23]. Thus, our observations suggest that the influence of EA on the change of ER expression in brain may be one of further mechanisms of EA normalizing the dysfunction of HPOA.

INT rats as experimental control we adopted were all of in the stage of

preestrus and estrus because the animal sexual hormes and brain ER expressions were changed with the sexual cycle [24]. All INT rats were selected to fix in the two stages there may be a relative constant comparability.

Our results show no same effects were seen after EA treatment in INT and following EA with control acupoints in OVX, suggesting that EA may possess a relative specificity on acupoint and the effect of EA may be a kind of normalization.

CONCLUSION

Our observations reveal that acupuncture may regulate the abnormal function of HPOA in many ways, which means that acupuncture may activate C-fos expression of brain, then a long term changes at molecular level would start, following the regulation of gene expression in FOS relative gene, such as ER mRNA and GnRH mRNA involved. On the other hand, EA may promote the activity of the body compensative mechanisms, then the levels of hormones, such as GnRH, LH, estrogen and so on would be normalized. The effect of acupuncture on regulating the function of HPOA may possess a relative specificity of acupoint. Moreover, our clinical and animal experimental results suggest that it is necessary for obtaining a satisfactory effect that proper stimulation should be about thirty minutes Q.D. for three days. This suggestion provides a successful consideration for clinical practice in curing the woman patients with dysfunction of sexual endocrine, such as primary ovarian dysfunction, climacteric syndrom, after-ovariectomy and polycystic ovarian disease etc.

ACKNOWLEDGMENT

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Acupuncture Treatment For Infertile Women Undergoing Intracytoplasmic Sperm injection

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ABSTRACT

Background Little information exists regarding the use of acupuncture in combination with allopathic treatment of infertility.

Objective To describe the use of acupuncture to stimulate follicle development in women undergoing in vitro fertilization.

Design, Setting, and Patients Prospective case series of 6 women receiving intracytoplasmic sperm injection and acupuncture along with agents for ovarian stimulation.

Main Outcome Measures Number of follicles retrieved, conception, and pregnancy past the 1st trimester before and after acupuncture treatment.

Results No pregnancies occurred in the non-acupuncture cycles. Three women produced more follicles with acupuncture treatment (mean, 11.3 vs 3.9 prior to acupuncture; P=.005). All 3 women conceived, but only 1 pregnancy lasted past the 1st trimester.

Conclusion Acupuncture may be a useful adjunct to gonadotropin therapy to produce follicles in women undergoing in vitro fertilization.

KEY WORDS

Female Infertility, Intracytoplasmic Sperm Injection, In Vitro Fertilization, Acupuncture

INTRODUCTION

Infertility is an area of women's health that has sparked much consumer interest in acupuncture. However, there is little published information concerning the combination of acupuncture with allopathic infertility technology.

We present results from 6 women treated with acupuncture to enhance follicle development during in vitro fertilization with intracytoplasmic sperm injection (ICSI) cycles. Our patients all had difficulty with follicle production despite maximum gonadotropin therapy. They were referred for acupuncture as a last resort. We compare results for the acupuncture cycle with results previous to acupuncture.

MATERIALS AND METHODS

The methods used for ovarian hyperstimulation have been described.1 Briefly, ovarian hyperstimulation was achieved using a long-acting gonadotropin-releasing hormone agonist (Lupron, TAP Pharmaceuticals Inc, Deerfield, Ill) administered either in the mid-luteal phase or following a minimum of 2 weeks of oral contraceptive treatment. After biochemical evidence of pituitary suppression (serum estradiol <40 pg/mL), subcutaneous follicle-stimulating hormone was given twice daily (3-6 amps/d). Follicular response was monitored with serial pelvic ultrasonography and serum estradiol measurements. When at least 2 follicles were >17 mm, 7500 IU of human chorionic gonadotropin was given intramuscularly, and transvaginal ultrasound-directed oocyte retrieval was scheduled 36 hours later. Oocytes were identified and then rinsed free of follicular fluid, blood, and debris in TALP-Hepes plus 10% serum substitute supplement (SSS) before being placed in 0.9 mL of bicarbonate-buffered human tubal fluid (HTF) medium plus 10% SSS.2 Spermatozoa were prepared using a discontinuous Percoll gradient. Oocytes for injection were denuded of cumulus cells using hyaluronidase followed by mechanical removal and then assessment for maturity. Metaphase II oocytes were injected with a single immobilized sperm.

Following ICSI, oocytes were cultured in 0.9 mL of HTF plus 10% SSS in organ culture dishes and housed in individually gassed chambers at 37ºC with 5% CO2, 5% O2, and 90% N2. At 15-18 hours following insemination, oocytes were assessed for pronuclei as evidence of fertilization. On the morning of day 3, cleaving embryos were transferred to 50-µL drops of S2 (Scandinavian IVF Sciences, Gothenburg, Sweden) under oil. Embryos of similar quality were grouped together. Embryos cultured beyond day 5 were transferred to fresh medium.

Luteal support consisted of intravaginal progesterone (300 mg/d) beginning on the day following embryo transfer in combination with 1500 IU of hCG intramuscularly given 5 days after oocyte retrieval.

Embryo transfer was performed on day 5 or 6 of extended culture using a Soft-Pass catheter (Cook Ob-Gyn, Bloomington, Ind).

The women began acupuncture treatment at the same time that they began follicle-stimulating hormone injections. They had 3 or 4 twice-weekly treatments, on days 1-3, 4-6, 7-9 and in some cases 9-11, with the final treatment on the day of or prior to egg retrieval.

Acupuncture treatments were aimed at stimulating Ming Men (BL 23, GV 4), Chong Mo, and Ren Mo. Points BL 23 and GV 4 were used at all treatments, whereas the Chong Mo (SP 4, MH 6) and Jenn Mo (KI 6, LU 7) Master and Couple points were alternated. Additional points were added on an individual basis, including LR 3, CV 4, 6, SP 30, BL 18, 20, 60, and 62.

Main outcome measures included the number of follicles retrieved, the incidence of pregnancy, and pregnancy lasting past the 1st trimester. Statistical analyses were calculated using SPSS version 10 (SPSS Inc, Chicago, Ill).

RESULTS

Results are shown in Table 1. None of the women achieved pregnancy during the non-acupuncture cycles. Three of the women (patients 1-3) clearly recruited more follicles with acupuncture than prior to acupuncture. For the 3 who responded, the mean number of follicles with acupuncture was 11.3 vs 3.9 prior to acupuncture (P=.005). All 3 achieved chemical pregnancy, but only 1 continued the pregnancy past the 1st trimester.

Patient 4 recruited fewer follicles during the acupuncture cycle than during previous cycles. Patients 5 and 6 recruited more follicles with acupuncture, but still recruited few follicles (P=.13). Patient 6 did achieve a chemical pregnancy, whereas patient 5 had the retrieval cancelled due to too few follicles.

On average, significantly more follicles were recruited with acupuncture than without (P=.02). Data on estrogen levels and endometrial lining thickness were not routinely collected in all cycles. For the 4 women (patients 1, 3, 4, and 5) who had estradiol levels measured during both acupuncture and non-acupuncture cycles, mean estradiol levels were higher during the acupuncture cycles than the non-acupuncture cycles (mean [SD], 1471 [480] pg/mL for acupuncture vs 731 [505] pg/mL for non-acupuncture), but this finding did not reach statistical significance (P=.08). Three women

(patients 1, 3, and 6) had endometrial lining measurements recorded for both acupuncture and non-acupuncture cycles. The difference in average endometrial lining thickness, measured on the day of follicle retrieval, did not approach statistical significance (acupuncture, 10.4 [2.2] mm vs non-acupuncture, 12.1 [1.1] mm, P=.33).

None of the 6 women reported any adverse reaction to the acupuncture treatments. There were no adverse reactions from the follicle retrievals or embryo transfers during either acupuncture or non-acupuncture cycles.

Table 1. Outcomes for Acupuncture vs Non-Acupuncture Cycles Among 6 Women Undergoing ICSI*

Patient No.	Age, y	Non-Acupuncture Cycles		Acupuncture Cycles		[redacted]
[redacted]	[redacted]	Follicles	Cycles	Follicles	Cycles	[redacted]
[redacted]	[redacted]	Mean No.	No.	Mean No.	No.	Outcome
1	29	4.7	3	8	1	IUP
2	34	2	1	10	2	SAB twice
3	36	3	2	14	1	SAB
4	37	8	1	6	1	No pregnancy
5	38	1	1	4	1	Cycle canceled
6	41	2	1	6	1	SAB
Mean (SD)		3.7 (1.0)		8.4 (1.3)		

*ICSI indicates intracytoplasmic sperm injection; IUP, intrauterine pregnancy; and SAB, early spontaneous abortion. P=.02 for overall acupuncture follicles vs non-acupuncture follicles.

DISCUSSION

Our findings suggest that acupuncture may be a useful adjuvant to gonadotropin therapy among women undergoing ICSI. In this context, acupuncture increased the number of follicles produced and appeared to also increase the estradiol level, but did not appear to affect

endometrial lining thickness. However, none of the women in this report had difficulty with achieving adequate endometrial lining.

Although there is significant consumer interest in using alternative and complementary therapies for infertility, there is little research that addresses the combination of techniques. Stener-Victorin et al3 published a report of using acupuncture to decrease the uterine pulsatility index among women with a history of poor uterine lining response to in vitro fertilization. They demonstrated a significant decrease in uterine pulsatility index, which was maintained for 2 weeks, by using 4 set acupuncture points with electric stimulation. Gerhard and Postneek4 published results of infertile women treated with acupuncture vs similar women treated hormonally, and showed a similar pregnancy rate among the 2 groups. Siterman et al5 showed improvement in sperm quality among subfertile men treated with acupuncture.

The mechanisms responsible for the systemic actions of acupuncture have been debated but not yet clearly defined. Traditional Chinese Medicine (TCM) speaks to increasing and harmonizing Qi within the reproductive organs.6 Scientific analysis of acupuncture used in the context of pain syndromes has shown acupuncture to raise the level of endogenous opiates7 and to decrease the level of sympathetic nerve stimulation8 at the painful area. The decrease in sympathetic stimulation may be 1 of the factors that results in an increased level of blood flow to the area.7,8 In the context of infertility, acupuncture may be helpful by increasing blood supply to the reproductive organs, or may simply increase relaxation or reduce subjective stress surrounding the infertility diagnosis and treatment.

Study Limitations

These cases have an obvious bias. The group was selected from those who responded poorly to gonadotropin therapy. The patients served as their own historical controls, but there was no similar group that simply had another ICSI attempt without acupuncture to compare before and after results. The acupuncture treatments were not standardized. Even though similar points were chosen for all women, points based on the individual TCM diagnosis were also used.

CONCLUSION

The cases do present evidence that a structured clinical trial of acupuncture to assist in follicle development for women undergoing in vitro fertilization and/or ICSI would be of interest. Many women

undergoing infertility treatment seek alternative care; knowing the interaction of these 2 systems would be most useful.

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Effects of Electro-Acupuncture on Nerve Growth Factor and Ovarian Morphology in Rats with Experimentally Induced Polycystic Ovaries

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ABSTRACT

Despite extensive research on the pathogenesis of polycystic ovary syndrome (PCOS), there is still disagreement on the underlying mechanisms. The rat model for experimentally induced polycystic ovaries (PCO)--produced by a single injection of estradiol valerate--has similarities with human PCOS, and both are associated with hyperactivity in the sympathetic nervous system. Nerve growth factor (NGF) is known to serve as a neurotrophin for both the sympathetic and the sensory nervous systems and to enhance the activity of catecholaminergic and possibly other neuron types. Electro-acupuncture (EA) is known to reduce hyperactivity in the sympathetic nervous system. For these reasons, the model was used in the present study to investigate the effects of EA (12 treatments, approximately 25 min each, over 30 days) by analyzing NGF in the central nervous system and the endocrine organs, including the ovaries. The main findings in the present study were first, that significantly higher concentrations of NGF were found in the ovaries and the adrenal glands in the rats in the PCO model than in the control rats that were only injected with the vehicle (oil or NaCI). Second, that repeated EA treatments in PCO rats resulted in concentrations of NGF in the ovaries that were significantly lower than those in non-EA-treated PCO rats but were within a normal range that did not differ from those in the untreated oil and NaCI control groups. The results in the present study provide support for the theory that EA inhibits hyperactivity in the sympathetic nervous system.

adrenal, central nervous system, follicular development, hypothalamus, ovary, ovulation, pituitary, stress

INTRODUCTION

Polycystic ovary syndrome (PCOS), one of the most common causes of anovulation in women of reproductive age. is a complex endocrine and metabolic disorder [1]. Despite extensive research seeking the pathogenesis of PCOS, there is still disagreement on the underlying mechanisms. Different hypotheses of its pathophysiology have emerged, which indicates that the etiology is multifactorial and poorly understood.

Women with PCOS have an increased risk of endometrial cancer, hypertension, and type II diabetes, and they need some kind of long-standing treatment [2]. Traditional pharmacological treatment for ovulation induction is effective, but side effects such as superovulation are quite common. A previous clinical study on anovulatory women with PCOS showed that sensory stimulation (i.e., electro-acupuncture [EA]) affects endocrinological and neuroendocrinological parameters [3]. In addition, regular ovulations were induced in more than one-third of the women without negative side effects. These findings accord with previous reports [4-6] but do not enlighten underlying mechanisms. The mechanisms behind the beneficial effect of EA on PCOS in the human are difficult to study because tissue samples from the ovaries and the central nervous system (CNS) are for obvious reasons unobtainable. Studies on, for instance, neuropeptides in the gonads and the CNS would be possible to conduct in an animal model, provided that such a model exists.

Experiments on normal cycling rats have shown that exogenous estradiol valerate (EV), a long-acting estrogen, causes acyclicity and the formation of polycystic ovaries (PCO) [7, 8]. The changes include atretic antral follicles, follicular cysts with a well-developed theca cell layer, a diminished granulosa cell compartment, and luteinized cysts [7, 8]. Furthermore, the rats exhibited alterations in basal and pulsatile LH and FSH concentrations, changes in the pituitary response to GnRH, degenerative changes in the hypothalamus, altered opioid inhibitory tone on GnRH release, and high estradiol levels with a persistent pattern of constant estrus as assessed by vaginal smear [9, 10]. In addition, EV-induced PCO is associated with an increase in peripheral sympathetic outflow, evidenced by an increase in the release of norepinephrine (NE), an increase in ovarian NE content, and a decrease in the number of ß-adrenergic receptors in the ovarian compartments receiving catecholaminergic innervation [9-11]. Even if it is not possible to reproduce human PCOS using a rat model, it may provide important leads because a single injection of EV induces an

anovulatory state that shares many endocrinological and morphological characteristics of human PCOS [7-13]. Thus, comparisons between the rat PCO model and human PCOS must be interpreted with caution because rat PCO ovaries contain multiple follicular cysts, the structure of which does not replicate the follicular growth arrest found in human PCOS. Contrary to previously held notions, the granulosa cells in the follicles accumulating in the human ovary are not atretic. However, both human PCOS and EV-induced PCO in rats may be associated with hyperactivity in the sympathetic nervous system.

According to one theory, elevated concentrations of neurotransmitters found in women with PCOS and anovulation may be associated with psychological stress and with hyperactivity in the sympathetic nervous system [3, 12, 13]. That superior ovarian nerve transection restores estrus cyclicity and ovulatory capacity in rats with EV-induced PCO further supports the theories of sympathetic hyperactivity [9]. Other evidence of neuronal involvement is that ovarian sympathetic innervation is under trophic control by nerve growth factor (NGF) [14]. This is also supported by the fact that the expression of the genes that encode NGF and one of its receptors, the low-affinity NGF-receptor, was dramatically increased in the ovary 30 days after EV injection [11]. Ovarian NGF is principally synthesized in the cells of the follicular wall [15], which is the site where the sympathetic neurons project to the ovaries [14]. The increase in the synthesis of NGF and its receptor that precedes the formation of cysts suggests that after PCO has been induced by EV injection, the neurons innervating the ovary are subjected to an enhanced neurotrophic influence that contributes to their hyperactivation and to the maintenance of an abnormally elevated catecholaminergic tone in ovarian steroid secretions [9-11].

Aim of the Study

Because NGF is known to serve as a neurotrophin for both the sympathetic and the sensory nervous systems and to enhance the activity of catecholaminergic and possibly other neuron types [9, 11, 14, 16-22], and because EA is known to reduce hyperactivity in the sympathetic nervous system [23-25], the experimentally induced PCO model was used to study the effects of EA by analyzing NGF in the CNS and the endocrine organs, including the ovaries.

The first part of the present study investigated dose-response--the discovery of the exact dose of EV needed to produce fully developed polycystic ovaries. The second part of this study investigated

treatment with EA--what contribution NGF made to the etiology and maintenance of EV-induced PCO in rats and if and to what extent EA has an effect on NGF and ovarian morphology in experimentally induced PCO.

MATERIALS AND METHODS

Fifty-nine virgin adult cycling Sprague-Dawley rats (Möllegaard, Denmark) weighing 190-210 g and with regular 4-day estrous cycles were used. The rats were housed at 22°C, four to a cage, with free access to pelleted food and tap water and with a 12L:12D cycle for at least 1 wk before and throughout the experimental period. All rats received a single i.m. injection of either EV (Riedeldehaen, Germany), oil, or 0.15 M NaCI (Kabi Pharmacia AB, Sweden) and were anesthetized with enfluran (EFRANE, Abbott Scandinavia, Kista, Sweden) and killed by decapitation. The local Animal Ethics Committee at Göteborg University, Sweden approved the study.

Dose-Response

Twenty-seven rats were injected with one of two different doses of EV in an oil solution or with oil alone to ascertain the optimal dose for induction of PCO [8]. They were decapitated on three different occasions (15, 30, or 60 days after i.m. injection) to elucidate precisely when the ovaries display characteristic features of well-defined PCO [7, 8]. Nine rats received 2 mg EV in 0.2 ml oil/rat, nine rats 4 mg EV in 0.2 ml oil/rat, and nine rats 0.2 ml oil alone. Three rats per dose were killed on Day 15, three on Day 30, and three on Day 60.

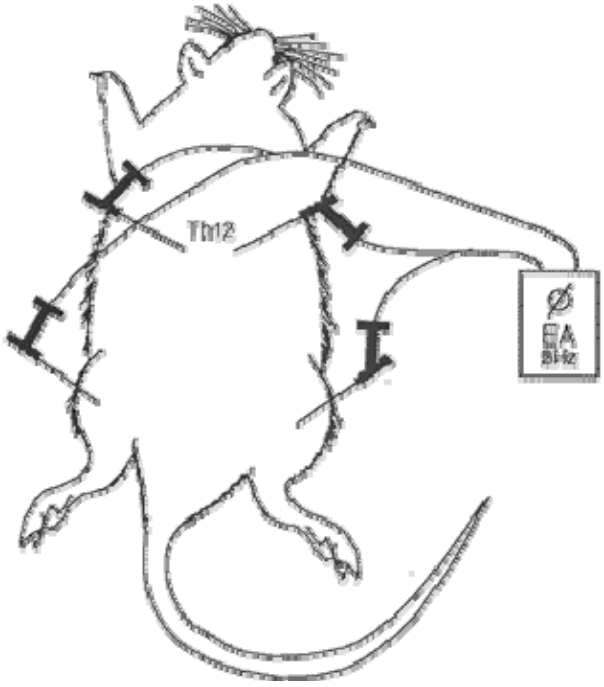

FIG. 1. Schematic drawing of the dorsal side of a rat and the placement of acupuncture needles. Two needles were placed bilaterally in the erector spinae muscle at the level of Th12 and two were placed in the quadriceps muscle bilaterally. The needles were then attached to an electrical stimulator for EA treatment.

Treatment with EA

The optimal dose (4 mg EV in 0.2 ml oil/rat) and timing (30 days after injection) were chosen for the experiments. In total, 32 rats took part. Eight rats in the EV control group and eight in the EA-treated EV group were injected i.m. with 4 mg EV in 0.2 ml oil/rat, eight rats in the oil control group with 0.2 ml oil, and eight rats in the NaCI control group with 0.2 ml 0.15 M NaCI. All 32 were decapitated on Day 30 after injection, that is, 1-2 days after the last EA treatment. All groups were anesthetized 12 times for about 25 min each time. Anesthesia was induced by inhalation of enfluran at 5.5-6.5 ml/h with an O2 and air flow of 0.25 L/min. The EA-treated EV group was subjected to 12 EA treatments every second or third day, beginning 2 days after the i.m. injection of EV. The stimulation points were bilateral in the quadriceps and erector spinae muscles at the level of thoracic (Th) 12 in the somatic segments according to the innervation of the ovaries (Th 12-lumbar [L]2, sacral [S]2-S4) (Fig. 1). The needles (Hegu; Hegu AB, Landsbro, Sweden) were inserted to depths of 0.5-0.8 cm and then bilaterally attached to an electrical stimulator (CEFAR ACU II, Cefar,

Lund, Sweden) with a low burst frequency of 2 Hz. Individual pulses within the frequency were square wave pulses with alternating polarities and with a pulse duration of 0.2 msec, 80 pulses/sec. The intensity was adjusted so that local muscle contractions were seen to reflect the activation of muscle-nerve afferents (A delta fibers and possibly C fibers) [26, 27]. The location and type of stimulation were the same in all rats.

Nerve Growth Factor Measurements by Enzyme Immunoassay

In the second part of the study, after the rats were decapitated, the pituitary gland, the hypothalamus, the hippocampus, one ovary, and the adrenal glands were quickly removed and dissected on dry ice, weighed, and stored at -80°C until extraction. The samples were sonicated in extraction buffer (0.1% Triton X-100, 100 mM Tris-HCI, pH 7.2, 400 mM NaCI, 4 mM EDTA, 0.2 mM PMSF, 0.2 mM benzethonium chloride, 2 mM benzamidine, 40 U/ml aprotinin, 0.05% sodium azide, 2% BSA, and 0.5% gelatin; 1 ml/100 mg of tissue), followed by centrifugation at 10,000 x g for 30 min. The supernatants were used for the assay. The bioactive form of 2.5S NGF purified from mouse sub-maxillary glands and prepared in the laboratory at the Institute of Neurobiology (CNR) in Rome, Italy, according to the method of Bocchini and Angeletti [28] was used as a standard. The NGF was dissolved in extraction buffer and the standard curve was in a range of 31.25 pg ml (-1) and 1 ng ml (-1). An ELISA was performed as described by Weskamp and Otten [29] with a minor modification [30]. Specific NGF binding was assessed by use of monoclonal mouse anti-ß-2.5S NGF (Boehringer Mannheim GmbH, Mannheim, Germany) that reacts with both the 2.5S and the 7S biologically active forms of NGF. The absorbency of samples and standards was corrected for nonspecific binding (i.e., the absorbency in a well coated with purified mouse IgG). The NGF content in the samples was determined in relation to the NGF standard curve. Data were not corrected for recovery of NGF from samples, which was routinely 70-90%, and was accepted only when the values were >2 SD above the blank. With these criteria, the limit of sensitivity of NGF ELISA averaged 0.5 pg per assay.

Morphology

One ovary per rat was removed, cleaned of adherent connective fat tissue, and fixed in 4% formaldehyde buffer; sections were stained with hematoxylin-eosin, and a trained pathologist performed a quantitative analysis of the follicle population. If ovum degeneration or

at least one pyknotic granulosa cell was seen, the follicle populations were classified as atretic, otherwise they were classified as healthy. Morphological characteristics of follicular atresia were, for instance, scattered pyknotic nuclei in the granulosa cell layer [31], detachment of the granulosa cell layer from the basement membrane [32], fragmentation of the basal lamina [33], and the presence of cell debris in the antrum of the follicle [34].

Statistical Analyses

Statistical analyses were carried out using the SPSS 8.0 software. The NGF concentrations in the pituitary gland, the hypothalamus, the hippocampus, the ovary, and the adrenal glands were analyzed and the groups compared using ANOVA followed by multiple comparison procedures (Bonferroni test). All results are presented as mean ± SEM. A P value less than 0.05 was considered significant. The 95% confidence interval (Cl) was given when $P < 0.05$.

RESULTS

Ovarian Morphology--Dose-Response

In the first part of the present study, dose-response, injection of 0.2 ml oil alone (control) was associated with a normal appearance of the ovaries and no differences were seen between rats sacrificed on Day 15, 30, or 60 (Fig. 2, a and b). No changes were seen in the ovaries of rats injected with 2 mg EV in 0.2 ml oil/rat and killed on Day 15. The ovaries of rats injected with the same dose of EV in oil exhibited small morphological changes resembling PCO when killed on Day 30 and 60 (Fig. 3, a and b). The ovaries of rats injected with a higher dose of EV (4 mg EV in 0.2 ml oil/rat) exhibited only small morphological changes on Day 15. Rats injected with the same dose of EV in oil and killed on Day 30 (Fig- 4, a-c) showed a progressive decrease in the number of primary and secondary follicles but it was on Day 60 (Fig- 5, a and b) that the true cystic follicles appeared and the well-defined PCO was fully developed in accordance with previous reports by Brawer et al. [8].

Ovarian Morphology--Treatment with EA

In the second part of the present study, treatment with EA, all rats were killed at Day 30 after EV injection, i.e., before the appearance of cystic follicles. The ovaries in the EV control group (4 mg EV in 0-2 ml oil/rat) displayed the same morphological changes as previously shown

in the dose-response section (see Fig- 4, a-c). The ovaries in the oil control group and the NaCI control group exhibited a typically normal appearance (see Fig- 2, a and b). No substantial morphological differences were found between the EA-treated, EV group, and the EV control group.

Nerve Growth Factor--Treatment with EA

In the second part of the present study, treatment with EA, NGF measurements were made at Day 30 after EV injection. Means ± SEM for NGF (pg/g wet weight) in the hypothalamus, the pituitary gland, the hippocampus, the ovary, and the adrenal gland in all groups are presented in Table 1. Ovarian NGF concentrations were significantly higher in the EV control group compared to the oil control group ($P < 0.001$, CI = 178.7, 821.6) and the NaCl control group ($P < 0.01$, CI = 144.6, 787.5). The NGF concentrations in the ovary were significantly lower in the EA-treated, EV group compared to the EV control group ($P < 0.05$ Cl = 6.2, 614.9) and did not differ from the (Jil and the NaCI control groups) The NGF concentrations in the adrenal glands were significantly higher in the EV control group and the EA-treated. EV group compared to both the oil control group ($P < 0.001$, CI = 45.7, 169.3 and $P < 0.01$, CI = 38.5, 166.5) and the NaCI control group ($P < 0.001$, Cl = 21.9, 162.9 and $P < 0.01$, Cl = 15.0, 159.8).

Weights of Ovaries and Adrenal Gland--Treatment with EA

Means ± SEM for weights (mg) of the ovaries and the adrenal glands in all groups are presented in Table 2. Ovarian weights in the control EV group and in the EV-treated EV group were significantly lower compared to the oil control group (both $P < 0.001$) and the NaCI control group (both $P < 0.001$).

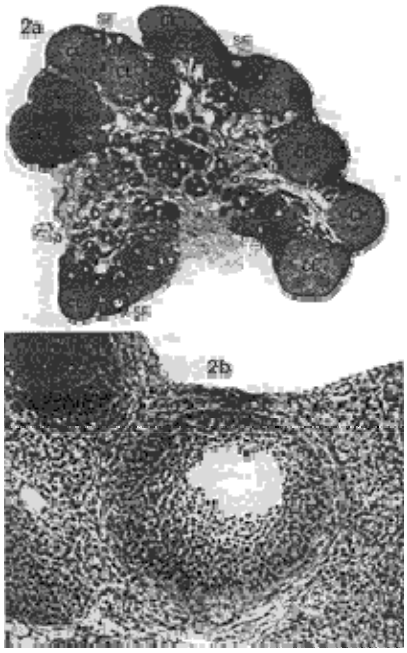

FIG. 2. **a)** Section of an ovary from a rat injected with 0.2 ml in oil and sacrificed on Day 30. In total, 11 corpora lutea (CL) marked with CL and three secondary follicles (SF) marked with SF are seen. One secondary follicle is framed (b). Magnification x2.5. Section stained with hematoxylin-eosin. **b)** Normal secondary follicle. Magnification x20.

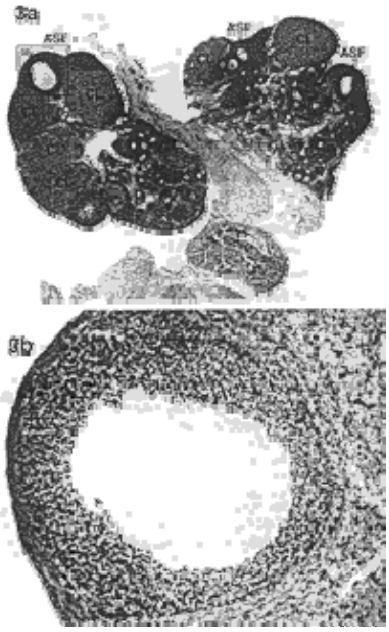

FIG. 3. **a)** Section of an ovary from a rat injected with 2 mg EV in 0.2 ml oil and killed on Day 30. In total, six corpora lutea marked with CL and three atretic secondary follicles (ASF) marked with ASF are seen. The atretic secondary follicle is framed (b): Magnification x2.5: Section stained with hematoxylin-eosin. **b)** An atretic secondary follicle with granulosa cells showing signs of atresia and intact theca cells. Magnification x20.

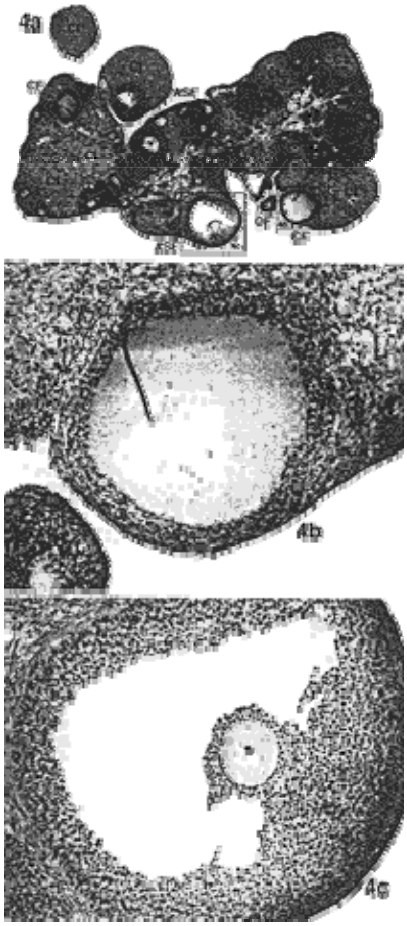

FIG. 4. **a)** Section of an ovary from a rat injected with 4 mg EV in 0.2 ml oil and sacrificed on Day 30. In total, seven corpora lutea marked with CL three cystic follicles (CF) marked with CF, and two atretic secondary follicles marked with ASF are seen. One cystic follicle (b) and one atretic secondary follicle are framed (c). Magnification x2 5: Section stained with hematoxylin-eosin. **b)** Cystic degenerating follicle showing a thin granulosa layer and debris in follicular fluid. Magnification x20. **c)** An atretic secondary follicle with detachment of the oocyte from the cumulus mass of pyknotic granulosa cells. Magnification x20.

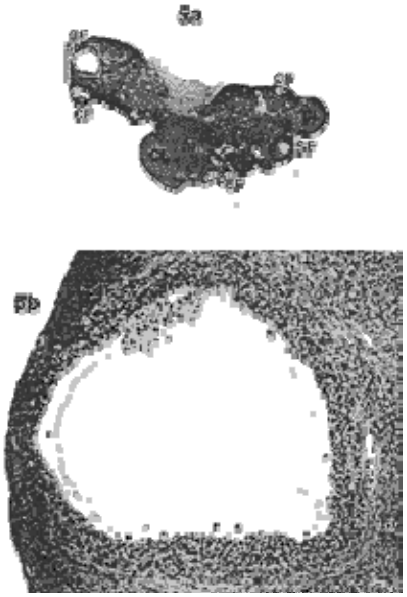

FIG. 5. **a)** Section of an ovary from a rat injected with 4 mg EV in 0.2 ml oil and sacrificed on Day 60. In total, two corpora lutea marked with CL, five cystic follicles marked with CF and one secondary follicle marked with SF are seen. One cystic follicle is framed (b). Magnification x2.5 Section stained with hematoxylin-eosin. **b)** A cystic degenerating follicle showing a thin granulosa layer and debris in follicular fluid. Magnification x20.

DISCUSSION

The main findings in the present study are as, follows: First, PCO induced in rats by a single injection or EV results in significantly higher concentrations of NGF in the ovaries and the adrenal glands without any changes in the brain tissue when measured 30 days after EV injection.

Second, repeated EA treatments with low frequency (2 Hz) significantly decrease the elevated NGF concentrations in the ovaries, to within a normal range, without affecting NGF concentrations in the adrenal glands or brain tissue when measured 30 days after EV injection.

The histological examination of the ovaries in the first part of the present study, dose-response, revealed that the optimal dose of EV that caused typical PCO-like morphological changes was 4 mg and that PCO was fully developed at Day 60. This dose was twice that used by Brawer and coworkers [7, 8] to achieve full development of a well defined PCO in rats. The reason might be differences in the strain of rat and/or the estrogen preparation that was used. In addition, the ovarian weight in the two EV-injected groups was significantly lower compared to that in the vehicle-injected (oil and NaCI) control rats. The reduction in ovarian weight and size, as well, are in accordance with the findings of Brawer et al. [8]. The reduction in weight and size of the ovaries might be explained by a reduction in the number of corpora lutea. In the second part, treatment with EA, no substantial influence in ovarian morphology was seen at Day 30, after EV injection with the number and duration of the EA treatments used in this study. However, the main reason for beginning EA treatment as early as 2-3 days after EV injection and to decapitate at Day 30 after EV injection was to estimate whether EA could influence the increased ovarian NGF concentrations that have been shown to precede the development of morphological changes in rats with PCO [11]. It remains to be shown whether EA influences the ovarian morphology 60 days after EV injection. It would therefore be of interest to study the effects of EA after extended treatment periods. Such a study would provide a unique opportunity to collect experimental evidence of the effectiveness of EA in humans. In fact, we have observed that the multifollicular pattern characteristic of the ovarian morphology of women with PCOS and anovulation, as assessed by ultrasonography, began to disappear after they had received repeated EA treatments [3].

An involvement of the nervous system in the etiology and/or maintenance of PCOS is suggested by both clinical and experimental findings [9-13]. Clinical studies show that women with PCOS temporarily recover normal ovarian function after bilateral wedge resection or ovarian drilling that partially denervates the ovary [35, 36]. There is thus a possibility that the ovarian nerves are involved in the successful outcome of bilateral wedge resection and ovarian drilling.

Experimental observations in rats reveal that superior ovarian nerve transection in EV-induced PCO reduces the steroid response, increases ß-adrenoreceptor concentrations to more normal levels, and restores estrus cyclicity and ovulation [9]. These effects were linked to reduced activity in the ovarian sympathetic nerve fibers, indicating a peripheral neurogenic effect [9].

Sensory stimulation, i.e., EA, activates muscle-nerve afferents, mainly A-delta and possibly C fibers [23, 26, 27], that initiate a number of peripheral reactions at the spinal level and centrally in the brain. That EA may reduce hyperactivity in the ovarian peripheral sympathetic nerve fibers is in accordance with the theory that EA could modulate sensory, motor, and autonomic outflow at the segmental level [24]. In parallel, higher control systems are activated, resulting in the release of a number of neuropeptides, important in the modulation of central and segmental autonomic outflow, of the hypothalamic-pituitary-ovarian axis (HPO axis), and of the descending pain-inhibiting systems [23-25].

TABLE 1. Treatment with EA.

	NGF concentration (pg/g)[a]			
	EA-treated, EV (n = 8)	EV control (n=8)	Oil control (n=8)	NaCI control (n=8)
Ovary	647.8 ± 69.9[b]	952.2 ± 95.1[d]	452 ± 42.4	486 ± 90.0
Adrenal gland	157.4 ± 11.8[c]	162.4 ± 22.1[e]	54.9 ± 9.7	70 ± 14.4
Pituitary gland	63.3 ± 9.6	95.5 ± 15.5	122.6 ± 36.2	125.8 ± 26.0

Hypothalamus	293.1 ± 26.8	293 ± 64.7	531.2 ± 155.7	315.6 ± 21.3
Hippocampus	3412.4 ± 210.2	3589.6 ± 292.2	2837.9 ± 122.7	3166.0 ± 164.8

[a] NGF (pg/g wet weight) in the ovary, the adrenal gland, the pituitary gland, the hypothalamus, and the hippocampus in the different groups: EA-treated, EV; EV control; oil control (0.2 ml); and NaCI control (0.2 ml 0.15 M). All EV iniections were 4 m8 EV In 0.2 ml oil/rat. All data values are mean ± SEM.
[b] $P < 0.05$, EA EV versus EV control.
[c] $P < 0.001$, EA EV versus oil control; and $P < 0.01$, EA EV versus NaCI control.
[d] $P < 0.001$, EV control versus oil control; and $P < 0.01$, EV control versus NaCI control.
[e] < 0.01, EV control versus oil control; and $P < 0.001$, EV control versus NaCI control.

TABLE 2. Treatment with EA.

	Weight (mg)[a]			
	EA-treated, EV (n = 8)	EV control (n=8)	Oil control (n=8)	NaCI control (n=8)
Ovary	0.011 ± 0.0007[b]	0.011 ± 0.0007[c]	0.021 ± 0.001	0.02 ± 0.001
Adrenal gland	0.018 ± 0.0008	0.016 ± 0.006	0.017 ± 0.001	0.018 ± 0.0011

[a] Weights of the ovary and the adrenal gland shown as mean ± SEM in the different groups: EA-treated, EV; EV control; oil control (0.2 ml); and NaCI control (0.2 ml 0.15 M). All EV injections were 4 mg EV in 0.2 ml oil/rat.
[b] $P < 0.001$, EA EV versus oil control and NaCI control.
[c] $P < 0.001$, EV control versus oil control and NaCI control.

For obvious reasons it is not possible to subject control animals to true sham needle insertion. As soon as a needle penetrates the skin, it may be seen as a form of sensory stimulation that activates afferent nerve fibers. If a sham needle insertion without electrical stimulation is

performed, then different acupuncture methods/stimulation techniques are being compared, and this does not provide proper information on the effect of EA versus no EA. We chose EA because the stimulation intensity is easy to standardize and it has been shown to be superior to manual needle stimulation [37]. In addition, to show a difference between two or more stimulation techniques would require a very large number of study subjects. In the present study, the control rats received the same enfluran anesthesia protocol as the rats treated with EA, which, in our opinion, is the best way to control completely environmental and/or emotional factors and the EA effect. The acupuncture needles in the present study were placed in the somatic segments that correspond to ovarian innervation. The needles were stimulated with low frequency EA for optimal activation of muscle nerve afferents to inhibit the autonomic outflow at the segmental level and at the central level and to modulate the HPO axis. The choice of acupuncture points and the aim of stimulation has been the same as in our other EA studies on the female reproductive tract that dealt with blood flow in the uterine arteries prior to in vitro fertilization (IVF) [38], pain-relief during oocyte aspiration in connection with IVF treatment [39], and induction of ovulation in women with PCOS [3].

We have shown that repeated EA treatments restore regular ovulations in more than one-third of the anovulatory women with PCOS. In addition, EA-influenced neuroendocrine and endocrine parameters indicative of PCOS, such as LH/FSH ratios, mean testosterone concentrations, and ß-endorphin concentrations, decreased significantiy [3]. The effects of repeated EA on anovulation were then attributed to an inhibition of hyperactivity in the sympathetic nervous system [3, 5, 6].

The findings of the present study support recent reports that ovarian NGF concentrations in rats with experimentally induced PCO [11] are elevated and that this increase can be related to a hyperactivity in the ovarian sympathetic nerves. Lara et al. [11] also suggests that activation of this neurotrophic-neurogenic regulatory loop is a component of the pathological process by which EV induces cyst formation and anovulation. They also stated that there is evidence that the alteration in neurotrophic input to the ovary contributes to the etiology and/or maintenance of human PCOS [11].

Furthermore, the present study shows that repeated EA treatments reduce peripheral sympathetic nerve hyperactivity, as revealed by the reduction in increased NGF concentrations in the ovaries into a normal

range 30 days after EV injection, that did not differ from that of the untreated oil and NaCI control groups.

It remains to be shown whether EA directly affects sympathetic nerve activity. Measurements of the nervous output by analyses of the catecholamine release can resolve this. In addition, because receptors for NGF are expressed on the endocrine cells of the ovary, activities of ovarian NGF may mediate and/or be mediated by alterations in endocrine factors, for example, by corticotropin-releasing hormone, prolactin, oxytocin, and/or adrenal corticosteroid secretion. To resolve this, the same experimental protocol regarding EA and controls used here must be supplemented with measurements of serum levels of these hormones.

Whether this condition can be reversed with EA treatment at higher stimulation intensities, in higher numbers, and/or over longer periods remains to be shown.

The conclusion of this study is that repeated EA treatments reduce ovarian NGF concentrations to within normal ranges. This suggests that EA inhibits the hyperactivity in the ovarian sympathetic nerves, which may be of importance for the development and maintenance of experimentally induced PCO.

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Substitution of Acupuncture for HCG in Ovulation Induction

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By using human menopausal gonadotropin (HMG) and human chorionic gonadotropin (HCG), fairly good clinical therapeutic efficacy has been obtained in the treatment of infertility. However, difficulties are brought about due to the ovarian hyperstimulation syndrome (OHSS) easily induced by these two drugs. Therefore, we attempted to use acupuncture instead of HCG in the induction of ovulation from 1989 to 1992, and satisfactory therapeutic effect was achieved as reported in the following.

General Data

Ten patients were hospitalized with confirmed diagnosis of infertility and totally observed for 11 menstrual cycles (one patient had recurrence of OHSS for 2 times). Their ages ranged from 27 to 30 years with an average of 29 years. After treatment by HMG, all patients manifested OHSS in varying degrees. In accordance with the criteria for grading of OHSS issued by WHO, among these 11 menstrual cycles 4 cycles were mild (ovarian slight enlargement less than 5 cm with symptoms of slight malaise of lower abdomen); 7 were moderate (marked enlargement of ovary with nausea, vomiting and abdominal distension); no severe case occurred (extreme enlargement of ovary with hydrothorax, ascites, pycnemia and electrolyte disturbance). In order to prevent the exacerbation of OHSS caused by combined use of HMG and HCG, acupuncture was used after HMG treatment to replace HCG for the ovulation induction in 11 menstrual cycles of these patients.

Therapeutic Method

1.5-3 *cun* long filiform needles (no. 28-30) were used. The acupoints used for needling were Zigong (Extra 16), Shenshu (UB 23), Ciliao (UB 32), (the above acupoints were used bilaterally) and Guanyuan (Ren 4). Baohuang (UB 53) and Zhongji (Ren 3) were selected according to the signs and symptoms as adjuvant points. The manipulation techniques included twirling, rotating, lifting and thrusting. Reinforcing

method was used in Shenshu point and the remaining points were punctured by reducing manipulation. The needling sensation should be transmitted toward both sides of lower abdomen. When arrival of Qi, retained the needles for 15 min. and manipulated the needles intermittently during the retaining period to enhance the stimulation. Moxibustion with moxa stick was used for some of these acupoints.

Observation of Therapeutic Effect

Criteria for assessment of therapeutic effect: Therapeutic effect was appraised mainly by comparison of ultrasonic B examination after needling with that before treatment and referred to the score of cervix uteri and basal body temperature to sit judgment on ovulation. Ovulation occurred within 24 h after 1st needling was considered as marked effect; ovulation within 72 h after 2-3 times of needling was effective; no ovulation occurred after 72 h after more than 3 times of needling was scored as ineffective.

Results of Treatment

Of the 11 menstrual cycles, marked effect was shown in 5 cycles, effective in 5 cycles and failed in 1 cycle. Among the 10 markedly effective and effective cycles, ovulation was induced in 2 cases after needling and diagnosed pregnancy by blood HCG assay and ultrasonography. In 9 of the 10 cycles treated with acupuncture for ovulation induction without using HCG and other drugs, the symptoms of OHSS were significantly remitted or even disappeared. Only in one cycle, HCG (with dosage less than for ovulation) was used after needling to maintain the function of corpus luteum and resulted in exacerbation of OHSS and finally remitted by drug treatment.

Typical Case

Fang, 27-year-old, suffered from polycystic ovary syndrome. She was unpregnant after married 2 years and the menstruation was only 1-2 times a year. The basal body temperature was monophase. No effect was observed using clomiphene and then treated with HMG. From the day 5, for bleeding due to withdrawal of progesterone, intramuscular injection of HMG was given at a dose of 150 U once a day for 8 days. The score of cervix uteri was 12 mark. The ultrasonogram showed that the size of right ovary was 9.6 cm x 7.8 cm x 4.6 cm and the left side was 9.2 cm x 7.2 cm x 4.7 cm. Both sides of ovary had 10-20 follicles with maximum size 1.8 cm. In order to avoid severe OHSS, acupuncture was used instead of HCG for ovulation induction after

stopping HMG treatment. On the next day after the first needling, the basal body temperature elevated from 36.3°C to 36.8°C and the score of cervix uteri fell from 12 mark to 9 mark, and ultrasonic B examination suggested that part of the follicles were ovulated. After the l9th day of ovulation, the blood concentration of HCG started rising and after 40 days the blood level of HCG reached to 35.6 ng/ml. The ultrasonogram showed that the diameter of embryonic sac was 1.5 cm and early pregnancy was diagnosed.

Discussion

It was reported in literature that using HMG-HCG in the induction of ovulation, the ovulatory rate was about 70%-90%, but the incidence of OHSS might be 10%-15.4% and even life-threatening in the severe case. At present, there were no satisfactory measures for the prevention and remission of OHSS. In most reports, it is considered that when OHSS inclines to occur, stopping injection of HCG is the effective way to avoid severe OHSS. However, stopping HCG would not only discontinue the ovulation of HCH, but also gave up the already developed follicles. Our clinical practice demonstrated that acupuncture is effective in ovulation induction and also the remission of OHSS induced by HMG. Furthermore, we also noted that in most OHSS patients enlarged ovaries and numerous developed follicles were revealed. As a result of excessive follicles developed, dysplasia of ova and insufficiency of corpus luteum often occurred, thus leading to uneasy pregnancy after ovulation. So it is reasonable to infer that using some Chinese drugs benefiting the function of corpus luteum or using certain amount of progesterone as supplementary treatment after acupuncture, the pregnancy rate could be raised.

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Relationship Between Blood Radioimmunoreactive Beta-Endorphin and Hand Skin Temperature During The Electro-Acupuncture Induction of Ovulation

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Abstract:

Thirteen cycles of anovulation menstruation in 11 cases were treated with Electro-Acupuncture (EA) ovulation induction. In 6 of these cycles which showed ovulation, the hand skin temperature (HST) of these patients was increased after EA treatment. In the other 7 cycles ovulation was not induced. There were no regular changes in HST of 5 normal subjects. The level of radioimmunoreactive beta-endorphin (rß-E) fluctuated, and returned to the preacupunctural level in 30 min. after withdrawal of needles in normal subjects. After EA, the level of blood rß-E in cycles with ovulation declined or maintained the range of normal subjects. But the level of blood rß-E and increase of HST after EA ($r=-0.677$, $P<0.01$). EA is able to regulate the function of the hypothalamic pituitary-ovarian axis. Since a good response is usually accompanied with the increase of HST, monitoring HST may provide a rough but simple method for prediciting the curative effect of EA. The role of rß-E in the mechanism of EA ovulation induction was discussed.

KEY WORDS: Electro-Acupuncture (EA), Hand Skin Temperature (HST), radioimmunoreactive beta-endorphin (rß-E), ovulation, radioimmunoassay (RIA)

INTRODUCTION

In our previous work, it has been demonstrated that EA is an effectual method of ovulation induction (1). The present work studied the relationship between the curative effect of EA and the changes of the HST and the level of blood beta-endorphin.

MATERIALS AND METHODS

Selection and Treatment of Cases

Eleven cases of chronically anovulatory patients including 9 cases of polycystic ovarian disease (PCO), 1 case of hypogonadotropic amenorrhea and case of oligomenorrhea were treated with EA in 13 menstruation cycles. They were 22 to 35 years of age and their courses of disease were 3 to 12 years. The basic body temperature (BBT) of these patients was monophase for at least 3 months. Each patient accepted the vaginal dropping cell examination twice or more a week. The results showed that the eosinocyte index (EI) of 10 cases was less than 30% and the EI of 1 case was more than 70%.

On the 10th day of each menstruation cycle, the patients were treated with EA. "Guanyuan" "Zhongji," "Sanyinjiao" and both sides of "Zigong" points were stimulated for 30 min. at 8:00 AM, OD for 3 days. The stimulation parameters were 7-10mA and 4-5HZ with G6805 model generator. The electric current of EA was bearable for every patient. Before and after the EA, HST was measured by a semiconduct thermometer and blood samples were collected from the forearm vien of patients for ß-E RIA. Five healthy woman voluteers with normal menstruation cycle were selected as controls. They were 31 to 35 years old and the menstruation cycle was 28 days. BBT showed change of biphase. All of them were healthy in premenorrhea and did not take any drug one month before EA. The stimulation points and parameters of EA were the same as above mentioned.

Plasma ß-Enorphin Radioimmunoassay

The blood samples were added to 100ug/ml bacitracin for inhibiting blood aminopeptidase and centrifuged at 3,000g for 15 min. The plasma was stored at -40°C.

The sensitive radioimmunoassay was performed as a routine in our lab (2,3), to determine the concentration of ß-E in the samples of plasma. Each estimative tube was added 0.1ml 1:8000 rabbit ß-E antiserum, 0.1ml[125]I-ß-E . That is 0.03ml sheep antiserum to rabbit gamma-

globulin diluted 20-fold with RIA buffer was added to each tube, than shaken and incubated at 0-4°C for 24 hours, and centrifuged at 3,000g for 15 min. The supernatant was poured out and the precipitate was counted for radioactivity in Model FH 408 gamma counter. ß-E contents were quantitated according to the standard curve which was performed at the same time with the sample tubes. The least detected quantity of RIA was 10pg/tube.

RESULTS

Clinical Observation

It was adopted standards of ovulation that BBT showed biphase and EI became cyclic variation. Six of 13 menstruation cycles treated with EA showed ovulation, while the other 7 cycles failed to do so. No EA effect was found in normal control subjects.

In the 13 anovulatary cycles, increased HST occurred in 6 cycles, of which 5 cycles showed ovulation after EA treatment. 7 cycles manifested decreased HST and only one of them produced ovulation (Table 1). No regular change was seen in HST in normal subjects.

Table l. Effect of EA Induction of Ovulation in 13 Cycles

Changes of HST	Ovulation	No Ovulation	Total
Increased	5*	1	6
Decreased	1	6	7

* P<0.05 as estimated by X[2] test

Change of Plasrna rß-E

In normal menstruation cycles the level of plasma rß-E before and after EA fluctuated and returned to the preacupural level after 30 minutes.

In the 13 anovulatory cycles the level of plasma rß-E on the 10th day of the cycles was higher but not statistically significant from that of normal subjects.

After EA the plasma rß-E contents of 6 cycles with ovulation either declined or maintained within the range of normal. And the plasma level of 7 cycles that failed to show ovulation after EA were significantly higher than those of normal subjects and 6 ovulatory cases as estimated by t test ($P<0.05$), (Table 2)

Table 2. Changes of blood ß-E level before and after EA* (pg/ml)

Group of cases	No. of cycles	Before EA	After EA
Ovulation	6	65.59 ± 24.15	38.86 ± 10.11
No ovulation	7	65.59 ± 24.15	80.09 ± 22.16**
Normal	5	38.84 ± 10.13	41.52 ± 6.40

*The values in this table are Mean ± SE
**$P<0.05$

Cycles which showed increase of HST after EA were associated with a declination of plasma rß-E Ievel but in cycles where HST decreased, the plasma rß-E level elevated after EA. There was a negative correlation between changes of plasma rß-E and HST as measured by rank correlation ($r=0.677$, $P<0.01$).

Discussion

According to our clinical practice of using EA to cure barreness, the curative effect was related to the changes of patients' HST. In general, provided that the body temperature was normal and the environmental temperature was constant round 25°C, the HST may reflect the state of sympathetic system of a patient.

From present results, it seems that the successful rate of EA ovulation induction was higher in patients with the depression of sympathetic activity. In normal subject whether HST increased or declined, no influence in ovulation was found. These results suggest that the relationship of ovulation and HST in normal women is different from that in anovulatory patients. Yen and his colleagues (4) first reported that enogenous opioid peptides can inhibit pituitary pulse secreting LH.

Fumiko, Akio and Michael reported in succession that morphine, ß-E and dynorphin can also depress LH pulse secretion (5,6,7). These substances may exert their action via regulating the secretion of LH-RH in hypothalmus. EA can affect the central opioid peptide level (2,8,9) thus it may regulate the function of hypothalamic-pituitary-ovarian axis via brain endogenous opiod peptides, such as ß-E and dynorphin etc.

In this study 11 cycles were PCO and the blood LH level in these cycles was marked higher than that of normal subjects. EA may promote the release of ß-E in the brain and reduce LH-RH secretion from hypothalamus. Therefore, the blood LH content released from the pituitary was decreased. This might be one of the mechanisms of EA ovulation induction.

The injection of ß-E into rat cerebellomedullary cisterm resulted in the increase of blood epinephrine (E), norepinephrine (NE) and dopamine (DA) levels, and there was a positive correlation in the dose of ß-E and the levels of blood E, NE, and DA (10). The result suggests that control ß-E may influence the activity of the sympathetic system. Our study showed that the sympathetic activity in normal subjects was not affected and the level of blood ß-E was relatively stable. Thus EA was not able to influence the normal ovulatory cycles. In anovulatory patients, especially in PCO cases, EA can depress sympathetic activity resulting in the increase of HST and the lowering the level of blood ß-E.

These results suggest that in anovulatory cases the hyperactive sympathetic system can be depressed by EA and the function of the hypothalamus-pituitary-ovarian axis can be regulated by EA via central sympathetic system. This might be another possible mechanism of EA ovulation induction.

Our study also suggest that measuring HST my provide a rough but simple method for predicting the effect of EA ovulation induction.

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Influence of acupuncture on the pregnancy rate in patients who undergo assisted reproduction therapy

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Objective: To evaluate the effect of acupuncture on the pregnancy rate in assisted reproduction therapy (ART) by comparing a group of patients receiving acupuncture treatment shortly before and after embryo transfer with a control group receiving no acupuncture.

Design: Prospective randomized study.

Setting: Fertility center.

Patient(s): After giving informed consent, 160 patients who were undergoing ART and who had good quality embryos were divided into the following two groups through random selection: embryo transfer with acupuncture (n = 80) and embryo transfer without acupuncture (n = 80).

Intervention(s): Acupuncture was performed in 80 patients 25 minutes before and after embryo transfer. In the control group, embryos were transferred without any supportive therapy.

Main Outcome Measure(s): Clinical pregnancy was defined as the presence of a fetal sac during an ultrasound examination 6 weeks after

embryo transfer.

Result(s): Clinical pregnancies were documented in 34 of 80 patients (42.5%) in the acupuncture group, whereas pregnancy rate was only 26.3% (21 out of 80 patients) in the control group.

Conclusion(s): Acupuncture seems to be a useful tool for improving pregnancy rate after ART. (Fertil Steril®2002;77:721- 4. ©2002 by American Society for Reproductive Medicine.)

Key Words: Acupuncture, assisted reproduction, embryo transfer, pregnancy rate

Acupuncture is an important element of traditional Chinese medicine (TCM), which can be traced back for at least 4,000 years. Acupuncture has been shown to alleviate nausea and vomiting, dental pain, addiction, headache, menstrual cramps, tennis elbow, fibromyalgia, myofascial pain, osteoarthritis, carpal tunnel syndrome, and asthma. Both physiologic and psychological benefits of acupuncture have been scientifically demonstrated in recent years.

However, so far there have been only a few serious trials concerning the use of acupuncture in reproductive medicine. Publications focus primarily on acupuncture therapy for male infertility (1, 2). Electroacupuncture may reduce blood flow impedance in the uterine arteries of infertile women (3). A positive impact of electroacupuncture on endocrinologic parameters and ovulation in women with polycystic ovary syndrome has been demonstrated (4). In addition, auricular acupuncture was successfully used in the treatment of female infertility (5). In the present study, we chose acupuncture points that relax the uterus according to the principles of TCM. Because acupuncture influences the autonomic nervous system, such treatment should optimize endometrial receptivity (6). Our main objective was to evaluate whether acupuncture accompanying embryo transfer increases clinical pregnancy rate.

Materials and Methods

This study was a prospective randomized trial at the Christian-Lauritzen-Institut in Ulm, Germany. It was approved by the ethics committee of the University of Ulm. A total of 160 healthy women undergoing treatment with in vitro fertilization (IVF; n = 101) or intracytoplasmic sperm injection (ICSI; n = 59) were recruited into the study. The age of the patients ranged from 21 to 43 (mean age: 32.5

= 4.0 years). The cause of infertility was the same for both groups (Table 1). Only patients with good embryo quality were included in the study. Using a computerized randomization method, patients were assigned into either the acupuncture group or the control group.

Table 1

Descriptive data on acupuncture and control group (mean ± SD or total number).

	Control group (n = 80)	Acupuncture group (n = 80)	Statistics Statistics
Age of patients (years)	32.1 ± 3.9	32.8 ± 4.1	NS
No. of previous cycles	2.0 ± 2.0	2.1 ± 2.1	NS
No. of transferred embryos	2.1 ± 0.5	2.2 ± 0.5	NS
IVF (n)	54	47	NS
ICSI (n)	26	33	NS
No. of cycles with male factor infertility	46	47	NS
No. of cycles with tubal disease	21	22	NS
No. of cycles with polycystic ovaries	2	2	NS
No. of cycles with unknown cause of infertility	11	9	NS
Endometrial thickness (mm)	9.9 ± 2.7	9.1 ± 2.4	NS
Plasma estradiol on day of embryo transfer (pg/mL)	1001 -± 635	971 ± 832	NS
Pulsatility index of uterine arteries (PI) before embryo transfer	2.00 ± 0.56	2.02 ± 0,45	NS
Pulsatility index of uterine arteries (PI) after embryo transfer	2.19 ± 0.52	2.22 ± 0,44	NS
Pregnant	21/80 (26.3%)	34/80 (42.5%)	*P*=.03

NS = not significant (P>.05).
Paulus. Acupuncture in ART. Fertil Steril 2002.

Ovarian stimulation, oocyte retrieval, and in vitro culture were performed as previously described (7). Transvaginal ultrasound-guided needle aspiration of follicular fluid was performed 36 to 38 hours after hCG administration. Immediately after follicle puncture, the oocytes were retrieved, assessed, and fertilized in vitro. Sperm preparation and culture conditions did not differ for either group.

In cases of severe male subfertility, ICSI was preferred, as described in the literature (8). Forty-eight hours after the IVF or ICSI procedure, embryos were evaluated according to their appearance as type 1 or 2 (good), type 3 or 4 (poor), as described in literature (9).

Just before and after embryo transfer, all patients underwent ultrasound scans of the uterus using a 7-MHz transvaginal probe (LOGIQ 400 Pro, GE Medical Systems Ultra-sound Europe, Solingen, Germany). Pulsed Doppler curves of both uterine arteries were measured by one observer. The pulsatility index (PI) for each artery was calculated electronically from a smooth curve fitted to the average waveform over three cardiac cycles.

A maximum of three embryos, in accordance with German law, were transferred into the uterine cavity on day 2 or 3 after oocyte retrieval. For embryo replacement, the patient was placed in a dorsal lithotomy position, with an empty bladder. The cervix was exposed with a bivalved speculum, then washed with culture media prior to embryo transfer. Labotect Embryo Transfer Catheter Set (Labotect GmbH, Go¨ ttingen, Germany) was used for atraumatic replacement owing to the curved guiding cannula with a ball end, allowing the set to be used reliably even with difficult anatomic conditions. The metallic reinforced inner catheter shaft al lowed safe passage through the cervical canal. When the catheter tip lay close to the fundus, the medium containing the embryos was expelled and the catheter withdrawn gently. After this procedure, the patient was placed at bed rest for 25 minutes. All oocyte retrievals and embryo transfers were performed by one examiner using the same method. The examiner was not aware of the patient's treatment group (control or acupuncture).

At the time of the embryo transfer, blood samples (10 mL) were obtained from the cubital vein. Plasma estrogen was determined by an immunometric method using the IMMULITE 2000 Immunoassay System (DPC Diagnostic Product Corporation, Los Angeles, CA).

Luteal phase support was given by transvaginal progesterone administration (Utrogest®, 200 mg, three times per day; Kade, Berlin, Germany). Progesterone administration was initiated on the day after oocyte retrieval and was continued until the serum ß-hCG measurement 14 to 16 days after transfer and, in cases of pregnancy, until gestation week 8.

Each patient in the experimental group received an acupuncture treatment 25 minutes before and after embryo transfer. Sterile disposable stainless steel needles (0.25 X 25 mm) were inserted in acupuncture point locations. Needle reaction (soreness, numbness, or distention around the point = Deqi sensation) occurred during the initial insertion. After 10 minutes, the needles were rotated in order to maintain Deqi sensation. The needles were left in position for 25 minutes and then removed. The depth of needle insertion was about 10 to 20 mm, depending on the region of the body undergoing treatment. Before embryo transfer, we used the following locations: Cx6 *(Neiguan)*, Sp8 *(Diji)*, Liv3 *(Taichong)*, Gv20 *(Baihui)*, and S29 *(Guilai)*.

After embryo transfer, the needles were inserted at the following points: S36 *(Zusanli)*, Sp6 *(Sanyinjiao)*, Sp10 *(Xuehai)*, and Li4 *(Hegu)*.

In addition, we used small stainless needles (0.2 X 13 mm) for auricular acupuncture at the following points, without rotation: ear point 55 *(Shenmen)*, ear point 58 *(Zhigong)*, ear point 22 *(Neifenmi)*, and ear point 34 *(Naodian)*. Two needles were inserted in the right ear, the other two needles in the left ear. The four needles remained in the ears for 25 minutes. The side of the auricular acupuncture was changed after embryo transfer. The patients in the control group also remained lying still for 25 minutes after embryo transfer. All treatments were performed by the same well-trained examiner, in the same way.

The primary point of the study was to determine whether acupuncture improves the clinical pregnancy rate after IVF or ICSI treatment. Student's t-test was used as a corrective against any possible imbalance between the two groups regarding the following variables: age of patient, number of previous cycles, number of transferred embryos, endometrial thickness, plasma estradiol on day of transfer, method of treatment (IVF or ICSI), and blood flow impedance in the uterine arteries (pulsatility index). Chi-square test was used to

compare the two groups. All statistical analyses were carried out using the software package Statgraphics (Manugistics, Inc., Rockville, MD).

Results

A total of 160 patients was recruited for the study. Patients who failed to conceive during the first treatment cycle were not reentered into the study. According to the randomization, 80 patients were treated with acupuncture, and 80 patients underwent the usual therapy without acupuncture.

As Table 1 shows, there were no statistically significant differences between the two groups with respect to the following covariants: age of patient, number of previous cycles, number of transferred embryos, endometrial thickness, plasma estradiol on day of transfer, or method of treatment (IVF or ICSI). Clinical indications for ART were the same for patients of both groups. The blood flow impedance in the uterine arteries (pulsatility index) did not differ between the groups before and after embryo transfer.

The analysis shows that the pregnancy rate for the acupuncture group is considerably higher than for the control group (42.5% vs 26.3%; P=.03).

Discussion

The acupuncture points used in this study were chosen according to the principles of TCM (10): Stimulation of *Taiying* meridians (spleen) and *Yangming* meridians (stomach, colon) would result in better blood perfusion and more energy in the uterus. Stimulation of the body points Cx6, Liv3, and Gv20, as well as stimulation of the ear points 34 and 55, would sedate the patient. Ear point 58 would influence the uterus, whereas ear point 22 would stabilize the endocrine system.

The anesthesia-like effects of acupuncture have been studied extensively. Acupuncture needles stimulate muscle afferents innervating ergoreceptors, which leads to increased ß-endorphin concentration in the cerebrospinal fluid (11). The hypothalamic ß-endorphinergic system has inhibitory effects on the vasomotor center, thereby reducing sympathetic activity. This central mechanism, which involves the hypothalamic and brainstem systems, controls many major organ systems in the body (12). In addition to central sympathetic inhibition by the endorphin system, acupuncture stimulation of the sensory nerve fibers may inhibit the sympathetic

outflow at the spinal level. By changing the concentration of central opioids, acupuncture may also regulate the function of the hypothalamic-pituitary-ovarian axis via the central sympathetic system (13).

Kim et al. (14) suggested that Li4 acupuncture treatment could be useful in inhibiting the uterus motility. In their rat experiments, treatment on the Li4 acupoint suppressed the expression of COX-2 enzyme in the endometrium and myometrium of pregnant and nonpregnant uteri.

Stener-Victorin et al. (3) reduced high uterine artery blood flow impedance by a series of eight electroacupuncture treatments, twice a week for 4 weeks. They suggest that a decreased tonic activity in the sympathetic vasoconstrictor fibers to the uterus and an involvement of central mechanisms with general inhibition of the sympathetic outflow may be responsible for this effect. In our study, we could not see any differences in the pulsatility index between the acupuncture and control group before or after embryo transfer. This may be due to a different acupuncture protocol and the selected sample of patients with high blood flow impedance of the uterine arteries (PI ≥ 3.0) in the Stener-Victorin et al. study.

As we could not observe any significant differences in covariants between the acupuncture and control groups, the results demonstrate that acupuncture therapy improves pregnancy rate.

Further research is needed to demonstrate precisely how acupuncture causes physiologic changes in the uterus and the reproductive system. To rule out the possibility that acupuncture produces only psychological or psychosomatic effects, we plan to use a placebo needle set as a control in a future study.

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Role of acupuncture in the treatment of female infertility

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Objective: To review existing scientific rationale and clinical data in the utilization of acupuncture in the treatment of female infertility.

Design: A MEDLINE computer search was performed to identify relevant articles.

Result(s): Although the understanding of acupuncture is based on ancient medical theory, studies have suggested that certain effects of acupuncture are mediated through endogenous opioid peptides in the central nervous system, particularly ß-endorphin. Because these neuropeptides influence gonadotropin secretion through their action on GnRH, it is logical to hypothesize that acupuncture may impact on the menstrual cycle through these neuropeptides. Although studies of adequate design, sample size, and appropriate control on the use of acupuncture on ovulation induction are lacking, there is only one prospective randomized controlled study examining the efficacy of acupuncture in patients undergoing IVF. Besides its central effect, the

sympathoinhibitory effects of acupuncture may impact on uterine blood flow.

Conclusion(s): Although the definitive role of acupuncture in the treatment of female infertility is yet to be established, its potential impact centrally on the hypothalamic-pituitary-ovarian axis and peripherally on the uterus needs to be systemically examined. Prospective randomized controlled studies are needed to evaluate the efficacy of acupuncture in the female fertility treatment. (Fertil Steril® 2002;78:1149-53. ©2002 by American Society for Reproductive Medicine.

Key Words: Acupuncture, female infertility, in vitro fertilization

Acupuncture as a therapeutic intervention has been extensively studied and is increasingly practiced in the United States. A recent survey of acupuncture released by an NIH Consensus Development panel (1) indicated that although there are inherent problems of design, sample size, and appropriate controls in the acupuncture literature, promising data exist for the use of acupuncture in treating nausea and vomiting (2), postoperative pain (3-5), addiction (6-9), and general pain syndromes (10-12). As a medical technique, acupuncture has also been reported as an adjunct in the treatment of various gynecologic problems (13-15).

Although conventional treatment options for female infertility have been well established, there have been few systematic reviews of complementary or alternative approaches to the treatment of infertility. In light of an increasing trend in the use of complementary and alternative medicine (16) and common inquiry and utilization of such approaches by patients suffering from infertility, we intend to review the existing scientific rationale and clinical data based on which acupuncture may exert an influence on the outcome of female fertility.

In examining the potential usefulness of acupuncture in enhancing female fertility, it is appropriate first to give some theoretical background for acupuncture. Although the theory of acupuncture stems from underlying traditional Chinese medicine premises that would define etiologies for infertility in terms of energy disturbance of imbalances, or organ deficiencies and excesses, we intend to review the existing literature by examining modern medical aspects of the central and peripheral modes of action of acupuncture as they impact on the hypothalamic-pituitary-ovarian axis and the pelvic organs, respectively. Moreover, the effect of acupuncture on anxiety and stress

and ensuing potential indirect effects on female fertility will also be discussed.

Background

Acupuncture is the manipulation of thin metallic needles inserted into anatomically defined locations on the body to affect bodily function. The US Food and Drug Administration has recently removed acupuncture needles from the category of experimental medical devices and now regulates them just like it does other devices, such as surgical scalpels and hypodermic needles, under good manufacturing practices and single-use standard of sterility (1).

The general theory of acupuncture is based on the premise that there are patterns of energy flow (Qi) through the body, which are essential for health. Disruption of this flow is believed to be responsible for disease. Acupuncture can correct imbalances of flow at identifiable points close to the skin.

According to the proposed international acupuncture nomenclature by The World Health Organization in 1991 (17), the meridian system consists of 20 meridians interconnecting about 400 acupoints. These acupoints correspond to specific areas on the surface of the body, which demonstrate higher electrical conductance because of the presence of higher density of gap junctions along cell borders. They act as converging points (or sinks) for electromagnetic fields. A higher metabolic rate, temperature, and calcium ion concentration, are also observed at these points. In principle, positive (anode) pulse stimulation of a point inhibits the organ function, whereas negative (cathode) pulse stimulation enhances that function (18). This forms the basis of electroacupuncture, which applies small electrical needles inserted in specific acupoints.

Effects of acupuncture on the hypothalamic-pituitary-ovarian axis and menstrual cycle

Although traditional Chinese medicine understanding of acupuncture is based on ancient medical theory, a modern and scientific neuroendocrine perspective has begun to evolve in the past two decades. Mayer et al. (19) first reported that acupuncture analgesia was induced through endorphin production and antagonized by the narcotic antagonist naloxone. Other studies similarly suggested that certain effects of acupuncture are mediated through the nervous

system, within which ß-endorphin and other neuropeptides have been implicated (20-22).

Acupuncture was shown by Petti et al. (20) to cause a significant increase in ß-endorphin levels during treatment, which lasted for up to 24 hours. ß-endorphin is derived from its precursor protein pro-opiomelanocortin, which is present in abundant amounts in neuronal cells of the arcuate nucleus of the hypothalamus, pituitary, medulla, and in peripheral tissues including intestines and ovaries (23-25). Pro-opiomelanocortin cleaves to form adrenocorticotropic hormone and ß-lipoprotein. Further cleavage of ß-lipoprotein yields neuropeptides including ß-endorphin. Aleem et al. (26, 27) demonstrated the presence of immunoreactive ß-endorphin in follicular fluids of both normal and polycystic ovaries.

The influence on gonadotropin secretion and the menstrual cycle by endogenous opioid peptides is believed to be mediated by their action on GnRH secretion (28). The hypothalamic ß-endorphin center and the GnRH pulse generator, in fact, are both situated within the arcuate nucleus. Quigley et al. (29) first reported an increased opioid inhibition of LH secretion in hyperprolactinemic patients with pituitary microadenomas. Ching (30) and Orstead and Spics (31), respectively, showed that opioid peptides suppress GnRH release in rats and rabbits.

The role of these neuropeptides, including ß-endorphin, in the regulation of GnRH secretion in humans has recently been reviewed by Kalra et al. (32) and Pau and Spies (33). Rossmanith et al. (34) demonstrated the role of opioid peptides in the initiation of the mid-cycle LH surge in normal cycling women. Meanwhile, measurement of ß-endorphin in ovarian follicular fluid of healthy ovulatory women revealed much higher levels than that in circulating plasma (35). The highest level of ß-endorphin was noted to be in the preovulatory follicle.

Because acupuncture treatment impacts on ß-endorphin levels, which in turn affect GnRH secretion and the menstrual cycle, it is logical to hypothesize that acupuncture may influence ovulation and fertility. Animal studies have revealed that acupuncture treatment normalized GnRH secretion and affected peripheral gonadotropin levels (36, 37). Various investigators have shown that in normally ovulatory or anovulatory women, acupuncture also influenced plasma levels of FSH, LH, E2, and P (38-40). Acupuncture as a surrogate for hCG in ovulation induction was successfully used by Cai (41). Chen and Yu

(42) showed that electroacupuncture normalized they hypothalamic-pituitary-ovarian axis, and in another study Chen (43) reported that 6 of 13 anovulatory cycles responded to acupuncture treatment.

A series published from the University of Heidelberg in Germany (44) used auricular acupuncture on 45 infertile women suffering from ovulatory dysfunction such as oligomenorrhea and luteal phase defect. The control group received medical treatment including bromocriptine, dexamethasone, levothyroxine, clomiphene citrate (CC), and gonadotropin. Although the investigators concluded that resumption of ovulatory cycles occurred significantly more often in the acupuncture group compared to the control group, pregnancy rates were not different between the two groups. However, interpretation of study data was very difficult due to the heterogeneity of the patient population and treatment modalities. Moreover, seven pregnancies in the acupuncture group were actually achieved with hormone treatment 6 months after acupuncture was stopped.

Another study by Stenver-Victorin et al. (45) evaluated the use of electroacupuncture for ovulation induction on 24 oligo/amenorrheic women with polycycstic ovarian syndrome (PCOS). The percentage of ovulatory cycles in all subjects was shown to improve from 15% (in a total of 3 months before treatment) to 66% up to 3 months after treatment. Responsive patients were noted to have significantly lower body mass index (BMI), waist-to-hip circumference ratio, serum T concentration, serum T/sex hormone-binding globulin ratio, and serum basal insulin level. They suggested that, in these selected patients with PCOS, acupuncture could be considered as an alternative or adjunct to pharmacological ovulation induction.

A recent prospective randomized controlled study by Paulus et al. (46) compared pregnancy rates in a total of 160 patients undergoing IVG. Acupuncture was performed in 80 patients 25 minutes before and after ET. After controlling confounding variables, clinical pregnancy rate for the acupuncture group (42.5%) was significantly higher than the control group (26.3%).

Peripheral effects of acupuncture

In addition to the central modulation of the hypothalamic-pituitary-ovarian axis, the effects of acupuncture on the autonomic nervous system have been well documented (47). In the early 1980s, Yao et al. (48) reported long-lasting cardiovascular depression induced by acupuncture stimulation of the sciatic nerve in unanesthetized

hypertensive rats. In the human, acupuncture was also shown to be sympathoinhibitory. After acupuncture, sympathetic nerve activity as measured by norepinephrine level, skin temperature, blood pressure, and pain tolerance threshold was shown to be decreased (49).

Endometrial thickness, morphology, and uterine artery blood flow have been implicated as important parameters for success of implantation of human embryos (50-57). Despite conflicting results in the utilization of these parameters during various stages of treatment to predict outcome in IVF, it is generally believed that adequate endometrial thickness is required to optimize pregnancy rate. Because endometrial thickness is a function of uterine artery blood flow, Sher and Fisch (58) reported a novel method of using vaginal sildenafil in an attempt to improve uterine artery blood flow and endometrial development in patients undergoing IVF.

With its central sympathoinhibitory effect, acupuncture may contribute to reduce uterine artery impedance and therefore, increase blood flow to the uterus. In fact, Sterner-Victorin et al. (59) demonstrated this when they performed acupuncture in 10 infertile women who were down-regulated by GnRH analog to avoid the effect of endogenous hormone on the uterine artery blood flow.

Pulsatility index in the uterine artery and skin temperature (on the forehead and lumbosacral area) were evaluated in three time periods-before, right after, and 2 weeks after acupuncture treatment (twice a week for 4 weeks). Pulsatility index and skin temperatures were found to be significantly decreased and increased, respectively, both right after and 14 days after acupuncture treatment. This effect was hypothesized to be caused by central inhibition of sympathetic activity.

Acupuncture and stress reduction

It has been well documented that infertility causes stress (60-65), and stress reduction may, in turn, improve fertility (66). However, the relationship between stress and infertility is that of a vicious cycle. Social stigmatization, decreased self-esteem, unmet reproductive potential of sexual relationship, physical and mental burden of treatment, and the lack of control on treatment outcome are just some of the factors that can lead to psychological stress in any couple pursuing infertility treatment. In turn, stress may lead to the release of stress hormones and influence mechanisms responsible for a normal ovulatory menstrual cycle through its impact on the hypothalamic-pituitary-ovarian axis.

The use of acupuncture for reducing anxiety and stress possibly through its sympathoinhibitory property and impact on ß-endorphin levels has been reviewed (67, 68), and the efficacy of acupuncture in depression has also been studied (69). Because the pharmacological side effects of anxiolytic and antidepressant drugs on infertility treatment outcome are largely unknown, acupuncture may provide an excellent alternative for stress reduction in women undergoing infertility treatment.

Discussion

The practice of acupuncture to treat identifiable patho-physiological conditions has been a subject of intense research. The underlying physiologic mechanisms of acupuncture such as the release of opioids and other peptides in the central peripheral nervous system, and its inhibition of the sympathetic nervous system have been increasingly established. Promising results from credible trials have emerged for the use of acupuncture in treating various pain syndromes, substance abuse, and chemotherapy-induced nausea and vomiting.

Although the definitive role of acupuncture in the treatment of female infertility is yet to be established, its neuroendocrine effect on the hypothalamic-pituitary-ovarian axis and the preliminary clinical data reviewed here justifies further clinical trials to systematically examine the efficacy of acupuncture in treating various conditions related to female infertility such as ovulatory dysfunction associated with PCOS. The peripheral impact of acupuncture in improving uterine artery blood flow and hence endometrial thickness also provides encouraging data regarding its potential positive effect on implantation.

Whether these potential beneficial effects of acupuncture on the reproductive system can be translated into improving infertility treatment outcomes will eventually mandate randomized controlled studies of adequate design. Because acupuncture is nontoxic and relatively affordable, its indications as an adjunct in assisted reproduction or as an alternative for women who are intolerant, ineligible, or contraindicated for conventional hormone induction of ovulation deserves serious research and exploration.

Appropriate training, credentialing, and certification of acupuncture practitioners by state agencies can facilitate the integration of acupuncture into the treatment of female infertility, and healthcare in general. The NIH Consensus Conference (1) agreed that this is necessary to allow the public and other health practitioners to identify

qualified acupuncture practitioners. With the help of the US Department of Education, issues of training and licensure of non-physician and physician practitioners have been addressed. There is sufficient evidence to acupuncture's value to expand its use into conventional medicine and treatment of female infertility, and to encourage further studies of its underlying mechanisms as well as to establish its clinical value.

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Clinical Studies of Chiropractic during Pregnancy

Low Back Pain during Pregnancy

Bery G, Hammar M, Moller-Nielsen J et al. Obstet and Gynecol, 72:71-75, 1988.

In this study, researchers identified the sacroiliac joints of the pelvis (frequently "adjusted" by chiropractors) as being responsible for the majority of low back pain cases in pregnancy. This is due to the significant number of hormonal and biomechanical changes occurring in the pelvis during pregnancy. Researchers found 7/10 women were helped by spinal manipulation in this study.

Textbook on Chiropractic & Pregnancy

Fallon J. International Chiropractic Association. 1994 - Arlington, Virginia.

According to Dr. Fallon, author and internationally recognized "chiropractic pediatrician", statistics from her office "have demonstrated that chiropractic adjustments effectively reduce the average amount of time spent in labor." Her data shows a nearly 25% reduction in the average labor times in those receiving chiropractic care versus the generally accepted average labor time; and in women who had given birth in the past, there was a 33% reduction in average labor time.

Italian Study on Chiropractic & Pregnancy

Postpartum pain was relieved in 90 of 120 patients who received chiropractic adjustments.

AMA study shows that pregnant women under chiropractic care have easier pregnancy and delivery.

AMA records released in 1987 during trial in the U.S. District Court Northern Illinois Eastern Division, No 76C 3777. Irvin Hendryson, M.D. a member of the AMA board of trustees did a clinical study which

revealed that pregnant women who received chiropractic adjustments in their third trimester were able to carry to term and deliver children with more comfort. (This information was never released to the public or media. In the meantime, the AMA continued to attack chiropractic as "unscientific and dangerous."

Medical expert states that less painkillers needed during delivery
if patient under chiropractic care.

Freitag, P.
Expert testimony of P. Freitag, M.D., Ph.D.
comparing results of two neighboring hospitals.

A study was conducted in which chiropractic adjustments were incorporated during many patients' pregnancy. It revealed that the need for painkillers during delivery was reduced by half. This study was suppressed by the AMA at the time because it revealed chiropractic effectiveness.

Back pain during pregnancy and labor

Diakow PR, Gadsby TA, Gadsby JB, Gleddie JG, Leprich DJ, Scales AM.

J Manipulative Physiol Ther. 1991 (Feb); 14 (2): 116-11

84% of patients receiving spinal manipulative therapy reported relief of back pain during pregnancy. There was significantly less likelihood of back labor when spinal manipulative therapy was administered during pregnancy.

Sacroiliac subluxation:
a common, treatable cause of low-back pain in pregnancy

Daly JM, Frame PS, Rapoza PA. Fam Prac Res J 1991;11(2):149-159

A retrospective review of 100 consecutive pregnancies, involving 94 women receiving prenatal care at a rural western New York family practice, was conducted. Back pain was spontaneously reported to the

physician by 23 women in 23 pregnancies. Eleven of the 23 women met diagnostic criteria for sacroiliac subluxation. These criteria include absence of lumbar spine and hip pathology, pain in the sacral region, asymmetrical movement of the posterior superior iliac spines upon forward flexion, a positive pelvic compression test and asymmetry of the anterior superior iliac spines. A cohort of 11 women meeting criteria for sacroiliac subluxation was treated with rotational manipulation of the sacroiliac joints. After manipulative therapy, 10 of the 11 women (91%) had relief of pain and no longer exhibited signs of sacroiliac subluxation.