Effect of Acupuncture on Vascular Biomarkers and Psychological Well-Being of Women Undergoing In Vitro Fertilization (IVF)

Principal investigator
Jennifer Phy, DO
TTUHSC Dept. of OB-GYN
806-743-4256
jennifer.phy@ttuhsc.edu

Co-Investigator #1
Jaou-Chen Huang, MD
TTUHSC Dept. of OB-GYN
jaou-chen.huang@ttuhsc.edu

Co-Investigator #3
Samuel Prien, PhD
TTUHSC Dept. of OB-GYN
samuel.prien@ttuhsc.edu

Co-Investigator #4
Sheila Garos, PhD
Texas Tech University - Department of Psychology
sheila.garos@ttu.edu

Co-Investigator #5
Lindsay Penrose, PhD
TTUHSC Dept. of OB-GYN
lindsay.penrose@ttuhsc.edu

Project Abstract

Infertility affects 12% of couples in the United States. In vitro fertilization (IVF) is the most advanced treatment for infertility. Despite decades of improvements in hormonal drug therapy and laboratory techniques, IVF embryos continue to have low implantation potential (~30%/embryo). Therefore, it is not uncommon to transfer multiple embryos to the uterus in order to enhance success. As a result, approximately 30% of IVF pregnancies result in multiple gestation, which lead to higher morbidity and mortality of the mothers and infants. The best method to reduce multiple birth and associated morbidity and mortality is to transfer a single embryo with enhanced implantation potential. A recent meta-analysis of acupuncture for IVF showed it may augment IVF success by ~10%, although the underlying mechanism remains unclear. We hypothesize that acupuncture enhances IVF success by enhancing the perfusion of pelvic organs including the ovaries and the uterus. Since undergoing IVF treatment is a stressful process to most patients, we further hypothesize that acupuncture stabilizes the mind-body interaction by optimizing the psychological well-being of patients receiving IVF treatment.

Experimental Approach: (1) Participants: Women (ages 21 to 42) who seek IVF treatment at the Center for Fertility & Reproductive Surgery will be eligible for the study. Seventy women will be recruited and randomly assigned to either the intervention (acupuncture plus standard care) or the comparison (standard care alone) group. (2) Intervention: Participants in the intervention group will receive 3 acupuncture sessions during the project with the first treatment between day 6–8 of the stimulated IVF cycle, second treatment on the day of embryo transfer and the third treatment post-transfer, within 48 hours. Participants in the comparison group will receive no intervention but will receive standard care. (3) Measures: The primary outcome measures include prostacyclin and thromboxane vasoactive biomarkers. Secondary outcome measures include perceived stress levels. (4) Procedure: Upon IRB approval, an informed consent will be provided to all participants. Pre- and post- acupuncture urinary metabolites of prostacyclin and thromboxane will be assessed. A standardized Perceived Stress Scale will be administered before each acupuncture session for the study group, and before a 30-40-minute waiting period for the control group.

Background and Significance

Infertility affects approximately 1 in 8 couples (6.7 million women) in the United States (http://www.cdc.gov/nchs/fastats/infertility). Treatment for infertility is often expensive, time-consuming and stressful for the patient and her family. IVF is the most effective treatment for infertility, as it brings the oocytes and sperm together directly, provides an ideal early environment for embryo development and bypasses female infertility factors such as tubal occlusion, endometriosis and pelvic adhesions. However, the cost of IVF is often a limiting factor for families, especially noting that only 30% of embryos will successfully implant and result in pregnancy. Transferring multiple embryos may contribute to a higher chance of success, but this is complicated by a higher chance of multiple births (Pandian 2009). Multiple gestation poses a higher risk to both the mother and her pregnancy.

Despite advancements in embryo culture media, incubation systems and improved embryo transfer techniques, implantation of embryos is not always achieved. Therefore, women often seek alternative treatments to enhance their reproductive potential to maximize their chance of success. A recent meta-analysis of acupuncture for IVF showed improved success by ~10%, although the underlying mechanism remains unclear (Manheimer 2008).

Optimal blood supply to the uterus and ovaries are essential to the development of oocytes and the timely maturation of the uterine lining in preparation for embryo implantation. Enhancing perfusion of the pelvic organs is likely to result in improved clinical outcomes. The blood perfusion to organs is determined by local vascular tones, which in turn is controlled by the balance of two key vasoactive molecules, prostacyclin and thromboxane. The former relaxes the vascular smooth muscle, dilates blood vessels, and prevents platelet aggregation; the latter contracts the vascular smooth muscle, constricts blood vessels, and promotes platelets aggregation. Both thromboxane and prostacyclin are unstable: the former's half-life is 37 seconds and that of the latter is 60 minutes. Their urinary metabolites, thromboxane B_2 (TBX2), and 2, 3-dinor-TXB_2 (both from thromboxane), and 6-keto-prostaglandin F1 alpha (6-keto $PGF_1\alpha$), and 2, 3-dinor-6-keto-$PGF_1\alpha$ (both from prostacyclin) are very stable. The concentrations of these metabolites are fairly easily determined using commercially available EIA assay kits.

Acupuncture is a traditional Chinese medicine therapeutic approach involving the insertion and manipulation of needles in the body to stimulate specific points. In an overview of the use of acupuncture in gynecology, Napadow (2008) and colleagues stated that recent basic and clinical research has demonstrated that acupuncture regulates uterine and ovarian blood flow, and that the effect is most likely mediated as a reflex response via the ovarian sympathetic nerves, and that the response is controlled via the supraspinal pathways. Additionally, Al-Inany (2008) suggested acupuncture may stimulate the production of endogenous opioids, which may inhibit the central nervous system's outflow and the biological stress response.

Stress is known to have a negative effect on reproduction and perhaps the menstrual cycle, and often negatively affects success of fertility treatment. Acupuncture has been found to reduce stress by activating endogenous opioids and blocking stress-induced elevations of HPA hormones. A recent meta-analysis by the National Institutes of Health, Division of Complementary and Alternative Medicine (NIHCAM) showed acupuncture enhances in vitro fertilization (IVF) success by approximately 10%. A recent meta-analysis results showed that the pooled clinical pregnancy rate from all of the acupuncture groups was significantly higher than that from all of the control groups (P=0.04) (Zheng, 2012). Six systematic reviews (including five meta-analyses) have shown that acupuncture on the day of embryo transfer (ET) has improved pregnancy rates in IVF (Kang, 2011). None of the reviews call for a rejection of this intervention and all, in fact, call for more exploration of its value to enhance IVF cycles.

It is not clear how the benefit is attained. Thus we further hypothesize that acupuncture stabilizes the mind-body interaction by optimizing the psychological well-being of patients receiving IVF treatment.

Project Narrative

Hypothesis and Specific Aims

The purpose of this pilot study is to determine if acupuncture affects the vasoactive molecules (prostacyclin and thromboxane) in women undergoing IVF therapy. The investigators' overall hypotheses for this area of study, to be addressed with future research, are: (1) acupuncture augments IVF success by enhancing the perfusion of pelvic organs including the ovaries and the uterus, and (2) acupuncture reduces the levels of stress and optimizes mind-body interaction in individuals undergoing IVF.

Specific Aims:

(1) To measure the effects of acupuncture on key vasoactive urine molecules, i.e. prostacyclin and thromboxane, before and after acupuncture, and
(2) to assess the impacts of acupuncture on the psychological well-being of women undergoing IVF treatment.

SIGNIFICANCE OF THE STUDY

Results from the investigators' study will shed light on the mechanism by which acupuncture improves IVF outcomes. This may lead to other treatment strategies to improve success. Likewise, an established mechanism by which acupuncture improves IVF may lead to greater acceptance of this non-conventional treatment modality by the general population.

Research Design and Methods

This is a prospective, randomized, controlled study. Participants will be women in the Principal Investigator's practice seeking IVF. Participants will be randomized 1:1 into either the acupuncture group or the standard of care (no acupuncture) group. Each group will have 3 study visits during their 8-week IVF cycle.

Rationale for the Control Group:
A sham control group is not used because systematic review suggested that sham acupuncture controls may unnecessarily complicate the RCT evidence base given the nature of objective outcomes in IVF study (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3124624/).

Power analysis

With 35 patients allocated to each group, assuming the Student's T-test will be used to determine the differences between two independent samples, effect sizes $d \geq 0.6$ will be found statistically significant with alpha .05 and power .80. In practical terms, since the PSS validation samples showed a mean about 13.5 with variance=6.2, only averaged differences greater than 1.5 raw score points in PSS will be found statistically significant.

We plan to seek extramural funding in the future. We will use the data obtained from this pilot study to perform power analysis and to calculate the number of participants needed in future proposals.

Inclusion criteria:

Women between ages 21-42 years seeking IVF. Participants must be willing to undergo acupuncture and have no contraindications to needle insertion.

Exclusion criteria:

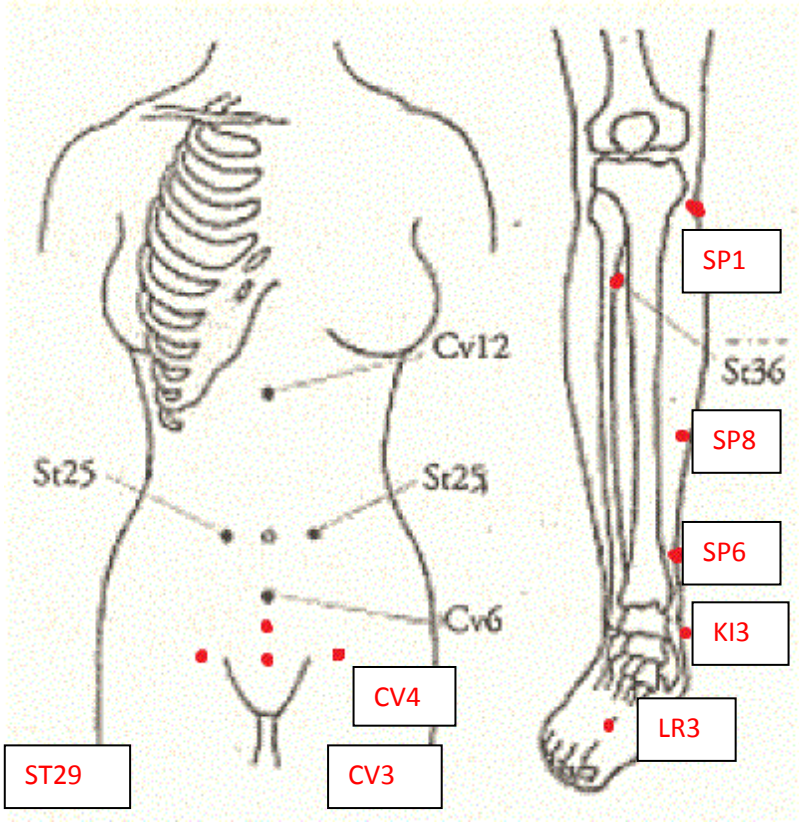
Women currently using alternative therapies such as acupressure, herbal supplements and meditation techniques will be excluded. Women with generalized psoriasis, neuropathy or coagulopathies posing increased risk due to needle insertion will also be excluded. Women must also agree not to take NSAIDs during their IVF treatment. Therefore women whom do not agree to avoid NSAIDs will be excluded from the study.

After fulfilling all requirements for inclusion in the study, patients will be randomized to the treatment group or the control group based upon random numbers generated before beginning the trial. The random numbers will be placed in consecutively numbered sealed envelopes.

Intervention

Acupuncture Protocol for treatment group

Figure1. Selected main acupoints for IVF protocol



An acupuncture treatment protocol will be followed using a Delphi consensus process developed specifically for patients undergoing IVF (Smith CA , Grant S , Lyttleton J , Cochrane S . 2012) . This Delphi consensus protocol is developed by17 international and national acupuncturists working in the fertility area. The points and timing were carefully chosen based on acupuncturists' experiences as well as research reviews. For instance, applying the third session of acupuncture on the day of transfer is supported by six systematic reviews (Kang, 2011).

Three semi-standardized sessions of manual acupuncture treatment will be administered during an IVF cycle including ET. Each session will run about 30~40 minutes at either the Family Medicine Clinic or Center for Fertility and Reproductive Surgery. The expense of all three sessions will be reimbursed by the project, NOT billed to the participant. The first treatment will be administered between days 6 through 8 of the stimulated IVF cycle, and will include core points ST29, CV4, CV6, SP6, SP10, plus up to 5 individualized additional points based on TCM pattern differentiation. The second treatment, prior to transfer on the day of embryo transfer, will use points SP8, SP10, LR3, ST29, CV4 and one selected from HT7/PC6/YinTang (depending on presentation of women). The third treatment to be done post-embryo transfer (any time following ET but before the pregnancy test) will use points GV20, KD3, ST36, SP6, PC6). This visit will occur within a windowed timeframe (within 48 hours post transfer, if possible, which includes anytime post-transfer on embryo transfer day) acknowledging the participant's ability to return during that timeframe. Main points are shown in Figure 1 and all points will be located based on WHO Standard Acupuncture Point Locations 2008 version. Up to 20 needles per session may be used. Disposable stainless-steel acupuncture needles (0.16×15 mm, 0.18 ×30mm and 0.20X40mm, DBC Spring Ten, Korea) will be used. Needles will be inserted manually to a depth varying from 10±5mm to 25±5 mm at the acupoints depending on the location and patient's physical figure. Needle retention time will be 25 minutes.

Auricular acupuncture using points Shenmen and Zigong only will be used on the day of ET. Needling sensation, *De qi*, is a composite of unique sensations interpreted as the flow of Qi or 'the arrival of vital energy'. *De qi* will be maintained with additional stimulation during the initial treatment on day 6–8, and during the pre-embryo treatment.

Control Group

The participants in the comparison group will continue their current usual IVF care at the Center for Fertility and Reproductive Surgery. Participants in the control group will also be asked to attend three study visits. The first visit will take place between days 6-8 of the IVF cycle. The second visit will take place on the day of embryo transfer, prior to transfer. The third visit will take place post-embryo transfer but before the pregnancy test. This visit will occur within a windowed timeframe (48 hours post transfer, which includes anytime post-transfer on embryo transfer day) acknowledging the participant's ability to return during that timeframe. At the beginning and end of each study visit, participants will provide a urine sample.

Study participants in this group will receive vouchers for 3 future acupuncture sessions, provided at no cost to them. These vouchers will be provided on the day of the participant's embryo transfer, or mailed to them after embryo transfer, and may be used beginning 1 year from the date of the embryo transfer. In the event that embryo growth does not progress or implantation is cancelled for any reason, the subject's participation in this study will end.
For those subjects vouchers will be mailed to them after study visit 1 and will be valid beginning one year from study visit 1. The vouchers will then be valid for 1 year. (i.e., Control group embryo transfer on 6-1-15. Patient receives 3 acupuncture vouchers that are valid 6-1-16 through 6-1-17). The delayed use of the acupuncture vouchers is intended to avoid use of the acupuncture sessions during pregnancy in the control group which could potentially affect outcome data comparisons between the two groups. For the same reason, subjects will be asked to commit to abstaining from acupuncture for the same period of 1 year post-embryo transfer, should they be randomized to the control arm. In event of error, vouchers may be replaced by mailing new ones to the subject.

Determination of Stable Metabolites of Prostacyclin and Thromboxane in the Urine

Urine samples will be collected from all study participants prior to and after each acupuncture session for the treatment group, and before and after each study visit for the control group. Stable metabolites of thromboxane and prostacyclin, 2, 3-dinor-TXB2 and 2, 3-dinor-6-keto-$PGF_1\alpha$, respectively will be determined on a later date. Specifically, a mid-stream urine sample will be collected in a sterile wide-mouth container. A 10mL aliquot will be centrifuged (300x *g*) for 10 min and the supernatant kept at -20C until assay. Stable metabolites of prostacyclin and thromboxane will be determined using ELISA kits according to the manufacturer's protocol: TXB2 ELISA kit (Enzo Life Sciences, Inc., http://www.biocompare.com/9956-Assay-Kit/1371009-TXBsub2sub-ELISA-kit/), Urinary prostacyclin ELISA kit (Enzo Life Sciences, Inc., http://www.enzolifesciences.com/ADI-900-025/urinary-prostacyclin-elisa-kit/). The specification of the kit shows that both kits have fairly high sensitivity. The lowest detection limits for TXB2 and prostacyclin metabolites are 10.54pg/mL and 6.58pg/mL, respectively. We plan to perform preliminary tests to determine the appropriate dilution of the samples before assay. In case the concentrations are below the detection limits, samples will be concentrated using a Centricon® Filter Concentrator (Millipore Co.). In case the above method cannot increase the concentration to within detectable ranges, GC/MS methodology will be used– we will consult collaborators (Ke-He Ruan, PhD, University of Houston) who determine prostacyclin and thromboxane metabolites using GC/MS on a routine basis.

Assessment of Self-Perceived Stress in Treatment and Control Group

To determine whether acupuncture contributes to stress reduction, study participants in the treatment group will complete the Perceived Stress Scale (Cohen, 1983 see Addendum) to assess self-perceived stress cycle days 6 through 8 of ovarian stimulation, on the day of embryo transfer, pre-embryo transfer and post-embryo transfer, but before the

pregnancy test, within 48 hours of transfer. Participants in this group will complete this scale before and after their acupuncture treatment.

Study participants in the control group will complete the same Perceived Stress Scale questionnaire once during days 6 through 8 of ovarian stimulation, on the day of embryo transfer, pre-embryo transfer and post-embryo transfer, but prior to the pregnancy test, within 48 hours of transfer.. In the event that the embryo transfer must be postponed for clinical reasons, the post-treatment questionnaire will be performed 3 to 7 days after the oocyte retrieval.

Study Data
The following data will be recorded for this study:

- Urine test collection date/time and results
- Acupuncture (control group wait) date/start/stop times
- Perceived Stress Scale data
- Age and race
- Pregnancy test results (+ pregnancy test (y/n), + clinical pregnancy (y/n)
- Pregnancy outcome (live birth (y/n))

Potential Problems and Alternative Approaches

Recruitment

Patients at the Center for Fertility and Reproductive Surgery will be screened for eligibility by Dr. Phy and Dr. Huang and, if eligible invited to take part in this study. We do not anticipate any problem recruiting enough individuals to participate in the investigators' study. The Texas Tech University Health Sciences Center, Center for Fertility & Reproductive Surgery currently performs approximately 70 IVF cycles per year. Even if 50% of patients participate in the study we have 70 patients in a 2 year period.

Poor Response to Egg Retrieval
Subjects whom are enrolled in the study who have a poor response to initial medication stimulation (identified early in the treatment cycle, warranting cancelling and new medication plan) can continue to be in the study if they plan on having retrieval at a later date. The subjects would be re-consented if changes were made to the ICF since the time they were last consented to be in the study. There would be no new randomization done. The subject would continue with their last randomization number and their previous ID. The subject will proceed in the study and continue with the study procedure visits #1- 3 when they return for future IVF.

Enzyme immunoassay of metabolites of prostacyclin and thromboxane:

In case the concentrations are below the detection limits, samples will be concentrated using a Centricon® Filter Concentrator (Millipore Co.). In case the above method cannot increase the concentration to within detectable ranges, GC/MS methodology will be used. We have collaborators (Ke-He Ruan, Ph.D., University of Houston) who determine prostacyclin and thromboxane metabolites using GC/MS on a routine basis.

Psychological testing
We do not anticipate any problem administering psychological testing. One of the investigators is an experienced clinician. She administered the proposed test many times in her career.

Removal from study
In the event that embryo growth does not progress or implantation is cancelled for any reason, the subject's participation in this study will end.

Risks
Participation in this study will not change the standard risks related to in vitro fertilization. Those who are randomized to the experimental group will undergo acupuncture. Acupuncture is generally considered safe when done by a trained professional. Risks of acupuncture may include occasional dizziness, bleeding, bruising, and potential anxiety. However, women who undergo IVF will perform up to 80-100 subcutaneous and intramuscular injections on themselves by the time of receiving acupuncture. Comparing to the bigger gauge (thicker hollow needle) of the syringe needles, discomfort caused by acupuncture needle will be similar but at a much lower level. Possible loss of confidentiality is a risk for all study participants, and will be mitigated by de-identifying all study data.

Benefits
Prior studies indicate a potential benefit of acupuncture on in vitro fertilization outcomes. However, the mechanism of the benefit is unclear. It is possible that taking part in this study will be of no benefit to the participant.

Confidentiality
Each subject will be assigned a unique identification number at the time of enrollment, and all data will be de-identified and labeled with the subjects' ID number. All de-identified data will be stored securely on encrypted devices accessible only to study investigators in accordance with TTUHSC policies.

Projected Timeline

Historically, patients seeking infertility treatment with IVF are open-minded to alternative therapy to enhance success. The TTUHSC Center for Fertility and Reproductive Surgery currently performs 70 IVF cycles per year. With a projected recruitment of 50% of women undergoing IVF, we anticipate reaching the investigators' goal of 70 subjects in a 2-year period.

Significance or Innovation of Expected Research Outcome

Acupuncture shows promise in enhancing success rates in women undergoing IVF. However, without an established mechanism of action, it is difficult to encourage patients to accept the treatment. Furthermore, understanding the mechanism of acupuncture's positive effect (i.e., increased vasoactive biomarkers and/or decreased perceived stress) may lead to the development of other treatment modalities to help infertile women. Finally, the general population may be more likely to accept this low-risk, affordable alternative treatment modality if we can provide a scientific explanation of the beneficial effects of acupuncture.

Specific Roles for Investigators

Principal Investigator, Dr. Jennifer Phy, is an Associate Professor and board certified Reproductive Endocrinologist in the Department of OB-GYN at TTUHSC, Lubbock. She will oversee the general progress of the project, recruit patients, ensure compliance with all regulations at the university and national level, and assist in manuscript preparation.

Co-Investigator #1, Dr. Jaou-Chen Huang, is a Professor and board certified Reproductive Endocrinologist in the Department of OB-GYN at TTUHSC, Lubbock. He will recruit patients, oversee proper collection of urine samples and assist in manuscript preparation.

Co-Investigator #2, Dr. Yan Zhang, is an Associate Professor and a licensed acupuncturist in the Department of Family Medicine at TTUHSC, Lubbock. She will perform acupuncture to participants and assist in manuscript preparation.

Co-Investigator #3, Dr. Samuel Prien, is a Professor, Director of Clinical and Research Laboratories, and IVF Laboratory Director in the Department of OB-GYN, TTUHSC, Lubbock. He will determine the levels of vasoactive biomarkers in the urine samples and assist in manuscript preparation.

Co-Investigator #4, Dr. Sheila Garos, is an Associate Professor of Psychology, Director of Counseling and Director of the Psychology Clinic at Texas Tech University, Lubbock. She will assist in interpretation of participant pre- and post-treatment questionnaires and assist in manuscript preparation.

Addendum:

PERCEIVED STRESS SCALE by **Sheldon Cohen**

The *Perceived Stress Scale* (PSS) is the most widely used psychological instrument for measuring the perception of stress. It is a measure of the degree to which situations in one's life are appraised as stressful. Items were designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives. The scale also includes a number of direct queries about current levels of experienced stress. The PSS was designed for use in community samples with at least a junior high school education. The items are easy to understand, and the response alternatives are simple to grasp. Moreover, the questions are of a general nature and hence are relatively free of content specific to any subpopulation group. The questions in the PSS ask about feelings and thoughts during the last month. In each case, respondents are asked how often they felt a certain way.

Evidence for Validity: Higher PSS scores were associated with (for example):

- failure to quit smoking
- failure among diabetics to control blood sugar levels
- greater vulnerability to stressful life-event-elicited depressive symptoms
- more colds

Health status relationship to PSS: Cohen et al. (1988) show correlations with PSS and: Stress Measures, Self-Reported Health and Health Services Measures, Health Behavior Measures, Smoking Status, Help Seeking Behavior.

Temporal Nature: Because levels of appraised stress should be influenced by daily hassles, major events, and changes in coping resources, predictive validity of the PSS is expected to fall off rapidly after four to eight weeks.

Scoring: PSS scores are obtained by reversing responses (e.g., 0 = 4, 1 = 3, 2 = 2, 3 = 1 & 4 = 0) to the four positively stated items (items 4, 5, 7, & 8) and then summing across all scale items. A short 4 item scale can be made from questions 2, 4, 5 and 10 of the PSS 10 item scale.

Norm Groups: L. Harris Poll gathered information on 2,387 respondents in the U.S.

Norm Table for the PSS 10 item inventory

Category	N	Mean	S.D.
Gender			
Male	926	12.1	5.9
Female	1406	13.7	6.6
Age			
18-29	645	14.2	6.2
30-44	750	13.0	6.2
45-54	285	12.6	6.1
55-64	282	11.9	6.9
65 & older	296	12.0	6.3
Race			
white	1924	12.8	6.2
Hispanic	98	14.0	6.9
black	176	14.7	7.2
other minority	50	14.1	5.0

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

Name _____Date_____

Age_____Gender (*Circle*): **M F** Other_____

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often
4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?	**0**	**1**	**2**	**3**	**4**
2. In the last month, how often have you felt that you were unable to control the important things in your life?	**0**	**1**	**2**	**3**	**4**
3. In the last month, how often have you felt nervous and "stressed"?	**0**	**1**	**2**	**3**	**4**
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	**0**	**1**	**2**	**3**	**4**
5. In the last month, how often have you felt that things were going your way?	**0**	**1**	**2**	**3**	**4**
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	**0**	**1**	**2**	**3**	**4**
7. In the last month, how often have you been able to control irritations in your life?	**0**	**1**	**2**	**3**	**4**
8. In the last month, how often have you felt that you were on top of things?	**0**	**1**	**2**	**3**	**4**
9. In the last month, how often have you been angered because of things that were outside of your control?	**0**	**1**	**2**	**3**	**4**
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	**0**	**1**	**2**	**3**	**4**

Please feel free to use the *Perceived Stress Scale* for your research.

References:

1. Lim, H., Paria, B. C., Das, S. K., Dinchuk, J. E., Langenbach, R., Trzaskos, J. M. and Dey, S. K. (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell 91, 197-208. PMID: 9346237.
2. 2002 National Survey of Family Growth, Key Statistics from the National Survey of Family Growth (http://www.cdc.gov/nchs/nsfg/abc_list_i.htm#infertility)
3. Anderson, R. E. (2010) A Woman's Age and Fertility. (http://www.socalfertility.com/age-and-fertility.html)
4. Pandian, Z., Bhattacharya, S., Ozturk, O., Serour, G. and Templeton, A. (2009) Number of embryos for transfer following in-vitro fertilisation or intra-cytoplasmic sperm injection. Cochrane Database Syst. Rev. 15, CD003416. PMID: 19370588.
5. Manheimer, E., Zhang, G., Udoff, L., Haramati, A., Langenberg, P., Berman, B. M. and Bouter, L. M. (2008) Effects of acupuncture on rates of pregnancy and live birth among women undergoing in vitro fertilisation: systematic review and meta-analysis. BMJ 336, 545-549. PMID: 18258932. PMCID: PMC2265327.
6. Anderson, B. J., Haimovici, F., Ginsburg, E. S., Schust, D. J. and Wayne, P. M. (2007) In vitro fertilization and acupuncture: clinical efficacy and mechanistic basis. Altern. Ther. Health Med. 13, 38-48. PMID: 17515023.
7. The Effect of Acupuncture on Infertility With In-Vitro Fertilization (IVF) Patients. (http://clinicaltrials.gov/ct2/show/NCT00317317?term=IVF+acupuncture&rank=1)
8. Huang, J. C., Wun, W. S., Goldsby, J. S., Egan, K., FitzGerald, G. A. and Wu, K. K. (2007) Prostacyclin receptor signaling and early embryo development in the mouse. Hum. Reprod. 22, 2851-2856. PMID: 17905746.
9. Huang, J. C., Goldsby, J. S. and Wun, W. S. (2004) Prostacyclin enhances the implantation and live birth potentials of mouse embryos. Hum. Reprod. 19, 1856-1860. PMID: 15205402.
10. Lim, H., Paria, B. C., Das, S. K., Dinchuk, J. E., Langenbach, R., Trzaskos, J. M. and Dey, S. K. (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell 91, 197-208. PMID: 9346237.
11. Ruan, K. H., Deng, H. and So, S. P. (2006) Innovative engineering of a single protein with cyclooxygenase and prostacyclin synthase activities directly converting arachidonic acid into the vascular protector prostacyclin. Biochemistry 45, 14003-14011. PMID: 17115695.
12. Majerus, P. W. (1983) Arachidonate metabolism in vascular disorders. J. Clin. Invest. 72, 1521-1525. PMID: 6313764.
13. Pace-Asciak, C. R. and Smith, W. L. (1983) Enzymes in the biosynthesis and catabolism of the eicosanoids: prostaglandins, thromboxanes, leukotrienes and hydroxy fatty acids. Enzymes 16, 544-604.
14. Samuelson, B., Goldyne, M., Granstrom, E., Hamberg, M., Hammarstrom, S. and Malmsten, C. (1978) Prostaglandins and thromboxanes. Ann. Rev. Biochem. 47, 997-1029. PMID: 209733.
15. Smith, W. L. (1986) Prostaglandin biosynthesis and its compartmentation in vascular smooth muscle and endothelial cells. Ann. Rev. Physiol. 48, 251-262. PMID: 3085582.
16. Funk, C. D. (2001) Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 294, 1871-1875. PMID: 11729303.
17. Ruan, K. H. (2004) Advance in understanding the biosynthesis of prostacyclin and thromboxane A2 in the endoplasmic reticulum membrane via the cyclooxygenase pathway. Mini Rev. Med. Chem. 4, 639-647. PMID: 15279598.

18. Ruan, K. H. and Dogné, J. M. (2006) Implications of the molecular basis of prostacyclin biosynthesis and signaling in pharmaceutical designs. Curr. Pharm. Des. 12, 925-941. PMID: 16533160.
19. Dogné, J. M., Hanson, J., de Leval, X., Pratico, D., Pace-Asciak, C. R., Drion, P., Pirotte, B. and Ruan, K. H. (2006) From the design to the clinical application of thromboxane modulators. Curr. Pharm. Des. 12, 903-923. PMID: 16533159.
20. Needleman, P., Turk, J., Jackschik, B. A., Morrison, A. R. and Lefkowith, J. B. (1986) Arachidonic acid metabolism. Annu. Rev. Biochem. 55, 69-102. PMID: 3017195.
21. Granstrom, E., Diczfalusy, U., Hamberg, M., Hansson, G., Malmsten, C. and Samuelson, B. (1982) Prostaglandins and the cardiovascular system (Oates, J. A., ed), pp. 15-58, Raven Press, New York.
22. Bunting S., Gryglewski, R., Moncada, S., and Vane J. R. (1976) Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac ateries and inhibits platelet aggregation. Prostaglandins 12, 897-913. PMID: 1005741.
23. Moncada, S., Herman, A. G., Higgs, E. A. and Vane, J. R. (1977) Differential formation of prostacyclin (PGX or PGI2) by layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. Thromb. Res. 11, 323-344. PMID: 335560.
24. Weksler, B. B., Ley, C. W. and Jaffe, E. A. (1978) Stimulation of endothelial cell prostacyclin production by thrombin, trypsin, and the ionophore A 23187. J. Clin. Invest. 62, 923-930. PMID: 361756.
25. Ingerman-Wojenski, C., Silver, M. J., Smith, J. B. and Macarak, E. (1981) Bovine endothelial cells in culture produce thromboxane as well as prostacyclin. J. Clin. Invest. 67, 1292-1296. PMID: 7014633.
26. Smith, W. L., DeWitt, D. L. and Allen, M. L. (1983) Biomodal distribution of the prostaglandin I_2 synthesis antigen in smooth muscle cells. J. Biol. Chem. 258, 5922-5926. PMID: 6406508.
27. Coceani, F., Ackerley, C., Seidlitz, E. and Kelsey, L. (2001) Function of cyclo-oxygenase-1 and cyclo-oxygenase-2 in the ductus arteriosus from foetal lamb: differential development and change by oxygen and endotoxin. Br. J. Pharmacol. 132, 241-251. PMID: 11156583.
28. Pakrasi, P. L. and Jain, A. K. (2008) Cyclooxygenase-2-derived endogenous prostacyclin reduces apoptosis and enhances embryo viability in mouse. Prostaglandins Leukot. Essent. Fatty Acids. 79, 27-33. PMID: 18771909.
29. Stener-Victorin, E., Waldenström, U., Andersson, S. A. and Wikland, M. (1996) Reduction of blood flow impedance in the uterine arteries of infertile women with electro-acupuncture. Hum. Reprod. 11, 1314-1317. PMID: 8671446.
30. Uchida, S. and Hotta, H. (2008) Acupuncture affects regional blood flow in various organs. Evid. Based Complement Alternat. Med. 5, 145-151. PMID: 18604254.
31. Zhang, S., Ye, X., Shan, Q., Zhang, W., Ye, L. and Cui, Y. (1999) Effects of acupuncture on the levels of endothelin, TXB2, and 6-keto-PGF1 alpha in apoplexy patients. J. Tradit. Chin. Med. 19, 39-43. PMID: 10453582.
32. Xu, N. (1993) Effect of electroacupuncture at "taixi" point on plasma thromboxane A2 and prostacyclin in the rabbit with renal ischemia. Zhen Ci Yan Jiu 18, 240-242. PMID: 7923725.
33. Yuan, J., Westney, O. L., Ruan, K. H. and Wang, R. (2009) A new strategy, SuperEnzyme gene therapy in penile rehabilitation. J. Sex Med. 6 Suppl 3, 328-333. PMID: 19267856.
34. Ruan, K. H., So, S. P., Cervantes, V., Wu, H., Wijaya, C. and Jentzen, R. R. (2008) An active triple-catalytic hybrid enzyme engineered by linking cyclo-oxygenaseisoform-1 to prostacyclin synthase that can constantly biosynthesize prostacyclin, the vascular protector. FEBS J. 275, 5820-5829. PMID: 19021758.
35. Ruan, K. H, So, S. P., Wu, H. and Cervantes, V. (2008) Large-scale expression, purification, and characterization of an engineered prostacyclin-synthesizing enzyme with therapeutic potential. Arch. Biochem. Biophys. 480, 41-50. PMID: 18835243.
36. Ruan, K. H., Wu, J. and Cervantes, V. (2008) Characterization of the substrate mimic bound to engineered prostacyclin synthase in solution using high-resolution NMR spectroscopy and

mutagenesis: implication of the molecular mechanism in biosynthesis of prostacyclin. Biochemistry 47, 680-688. PMID: 18081314.

37. Ruan, K. H., Deng, H., Wu, J. and So, S. P. (2005) The N-terminal membrane anchor domain of the membrane-bound prostacyclin synthase involved in the substrate presentation of the coupling reaction with cyclooxygenase. Arch. Biochem. Biophys. 435, 372-381. PMID: 15708381.
38. Ni, F., So, S. P., Cervantes, V. and Ruan, K. H. (2007) A profile of the residues in the second extracellular loop that are critical for ligand recognition of human prostacyclin receptor. FEBS J. 275, 128-137. PMID: 18042246.
39. Zhang, L., Bastepe, M., Jüppner, H. and Ruan, K. H. (2006) Characterization of the molecular mechanisms of the coupling between intracellular loops of prostacyclin receptor with the C-terminal domain of the Galphas protein in human coronary artery smooth muscle cells. Arch. Biochem. Biophys. 454, 80-88. PMID: 16942748.
40. Zhang, L., Wu, J. and Ruan, K. H. (2006) Solution structure of the first intracellular loop of prostacyclin receptor and implication of its interaction with the C-terminal segment of G alpha s protein. Biochemistry 45, 1734-1744. PMID: 16460020.
41. Ruan, C. H, Wu, J. and Ruan K. H. (2005) A strategy using NMR peptide structures of thromboxane A2 receptor as templates to construct ligand-recognition pocket of prostacyclin receptor. BMC Biochem. 4, 6-23. PMID: 16271145.
42. Zhang, L., Huang, G., Wu, J. and Ruan, K. H. (2005) A profile of the residues in the first intracellular loop critical for Gs-mediated signaling of human prostacyclin receptor characterized by an integrative approach of NMR-experiment and mutagenesis. Biochemistry 44, 11389-11401. PMID: 16114876.
43. Ruan, K. H., Wu, J., So, S. P. and Jenkins, L. A. (2003) Evidence of the residues involved in ligand recognition in the second extracellular loop of the prostacyclin receptor characterized by high resolution 2D NMR techniques. Arch. Biochem. Biophys. 418, 25-33. PMID: 13679079.
44. Ruan, K. H., Wu, J. and Wang, L. H. (2005) Solution structure of a common substrate mimetic of cyclooxygenase-downstream synthases bound to an engineered thromboxane A2 synthase using a high-resolution NMR technique. Arch. Biochem. Biophys. 444, 165-173. PMID: 16297851.
45. So, S. P., Li, D. and Ruan, K. H. (2000) Identification of the substrate interaction site in the N-terminal membrane anchor segment of thromboxane A2 synthase by determination of its substrate analog conformational changes using high resolution NMR technique. J. Biol. Chem. 275, 40679-40685. PMID: 11006279.
46. Ruan, K. H., Li, D., Ji, J., Lin, Y. Z. and Gao, X. (1998) Structural characterization and topology of the second potential membrane anchor region in the thromboxane A2 synthase amino-terminal domain. Biochemistry. 37, 822-830. PMID: 9454571.
47. Ruan, K. H., Cervantes, V. and Wu, J. (2009) Ligand-specific conformation determines agonist activation and antagonist blockade in purified human thromboxane A2 receptor. Biochemistry 48, 3157-3165. PMID: 19170518.
48. Ruan, K. H., Wijaya, C., Cervantes, V. and Wu, J. (2008) Characterization of the prostaglandin H2 mimic: binding to the purified human thromboxane A2 receptor in solution. Arch. Biochem. Biophys. 477, 396-403. PMID: 18590695.
49. Ruan, K. H., Cervantes, V. and Wu, J. (2008) A simple, quick and high-yield preparation of the human thromboxane A_2 receptor in full size for structural studies. Biochemistry 47, 6819-6826. PMID: 18529068.
50. Wu, J., Feng, M. and Ruan, K. H. (2008) Assembling NMR structures for the intracellular loops of the human thromboxane A2 receptor: implication of the G protein-coupling pocket. Arch. Biochem. Biophys. 470, 73-82. PMID: 18073117. PMCID: PMC2295216.
51. Ruan, K. H., Wu, J., So, S. P., Jenkins, L. A. and Ruan, C. H. (2004) NMR structure of the thromboxane A2 receptor ligand recognition pocket. Eur. J. Biochem. 271, 3006-3016. PMID: 15233797.

52. Geng, L., Wu, J., So, S. P., Huang, G. and Ruan, K. H. (2004) Structural and functional characterization of the first intracellular loop of human thromboxane A2 receptor. Arch. Biochem. Biophys. 423, 253-265. PMID: 15001390.
53. Wu, J., So, S. P. and Ruan, K. H. (2003) Solution structure of the third extracellular loop of human thromboxane A_2 receptor. Arch. Biochem. Biophys. 414, 287-293. PMID: 12781781.
54. So, S. P., Wu, J., Huang, G., Huang, A., Li, D. and Ruan, K. H. (2003) Identification of residues important for ligand binding of thromboxane A_2 receptor in the second extracellular loop using the NMR experiment-guided mutagenesis approach. J. Biol. Chem. 278, 10922-10927. PMID: 12551898.
55. Ruan, K. H., Cervantes, V. and So, S. P. (2009) Engineering of a novel hybrid enzyme: an anti-inflammatory drug target with triple catalytic activities directly converting arachidonic acid into the inflammatory prostaglandin E2. Protein Eng. Des. Sel. 22, 733-740. PMID: 19850676.
56. Chillar, A., Wu, J. So, S. P. and Ruan, K. H. (2008) Involvement of non-conserved residues important for PGE2 binding to the constrained EP3 eLP2 using NMR and site-directed mutagenesis. FEBS Lett. 582, 2863-2868. PMID: 18652829.
57. Huang, J. C., Arbab, F., Tumbusch, K. J., Goldsby, J. S., Matijevic-Aleksic, N. and Wu, K. K. (2002) Human fallopian tubes express prostacyclin (PGI) synthase and cyclooxygenases and synthesize abundant PGI. J. Clin. Endocrinol. Metab. 87, 4361-4368. PMID: 12213900.
58. Arbab, F., Goldsby, J., Matijevic-Aleksic, N., Huang, G. Ruan, K. H. and Huang, J. C. (2002) Prostacyclin is an autocrine regulator in the contraction of oviductal smooth muscle. Hum. Reprod. 17, 3053-3059. PMID: 12456602.
59. Huang, J. C., Goldsby, J. S., Arbab, F., Melhem, Z., Aleksic, N. and Wu, K. K. (2004) Oviduct prostacyclin functions as a paracrine factor to augment the development of embryos. Hum. Reprod. 19, 2907-2912. PMID: 15492023.
60. Huang, J. C., Wun, W. S., Goldsby, J. S., Matijevic-Aleksic, N. and Wu, K. K. (2004) Cyclooxygenase-2-derived endogenous prostacyclin enhances mouse embryo hatching. Hum. Reprod. 19, 2900-2906. PMID: 15489241.
61. Huang, J. C., Wun, W. S., Goldsby, J. S., Wun, I. C., Noorhasan, D. and Wu, K. K. (2007) Stimulation of embryo hatching and implantation by prostacyclin and peroxisome proliferator-activated receptor delta activation: implication in IVF. Hum. Reprod. 22, 807-814. PMID: 17114194.
62. Ruan, K. H. Patent article, (WO/2007/104000) HYBRID PROTEIN THAT CONVERTS ARACHIDONIC ACID INTO PROSTACYCLIN. The World Intellectual Property Organization (WIPO) 13.09.2007.
63. de Lacey, S., Smith, C. A. and Paterson, C. (2009) Building resilience: a preliminary exploration of women's perceptions of the use of acupuncture as an adjunct to In Vitro Fertilisation. BMC Complement Altern. Med. 9, 50. PMID: 20003370; PMCID: PMC2799389.
64. Ng, E.H., So, W. S., Gao, J., Wong, Y. Y. and Ho, P. C. (2008) The role of acupuncture in the management of subfertility. Fertil. Steril. 90, 1-13. PMID: 18440533.
65. Stener-Victorin, E. and Humaidan, P. (2006) Use of acupuncture in female infertility and a summary of recent acupuncture studies related to embryo transfer. Acupunct. Med. 24, 157-163. PMID: 17264833.
66. Ho, M., Huang, L. C., Chang, Y. Y., Chen, H. Y., Chang, W. C., Yang, T. C. and Tsai, H. D. (2009) Electroacupuncture reduces uterine artery blood flow impedance in infertile women. Taiwan J. Obstet. Gynecol. 48, 148-151. PMID: 19574177.
67. Shi, X., Li, J., Zhou, J., Han, J., Zhao, J., Bian, J., Li, Y., Zhang, H., Ma, C., Song, Q. and Zhao, J. A. Clinical Study on Acupuncture with "Xingnao Kaiqiao" Method for Treatment of Apoplexy (http://www.ontcm.com/dotnetnuke/NewsEvents/tabid/639/articleType/ArticleView/articleId/1734/A-Clinical-Study-on-Acupuncture-with-Xingnao-Kaiqiao-Method-for-Treatment-of-Apoplexy.aspx)

68. Clincal study on MAW patients with Electro-acupuncture as the Major Therapy. (http://www.latest-science-articles.com/Medical_health/Clincal-study-on-MAW-patients-with-Electro-acupuncture-as-the-Major-Therapy-5772.html)
69. Lifang Liang, O.M.D., Acupuncture and IVF, Published by Blue Poppy Press, First Edition, August 2003, ISBN 1-891845-24-1.
70. http://www.goacupuncture.com/cgi-bin/ie/Home-ie.pl
71. Huang, J. C., Wun, W. S., Goldsby, J. S., Wun, I. C., Falconi, S. M. and Wu, K. K. (2003) Prostacyclin enhances embryo hatching but not sperm motility. Hum. Reprod. 18, 2582-2589. PMID: 14645174.
72. Urbich, C., Kuehbacher, A. and Dimmeler, S. (2008) Role of microRNAs in vascular diseases, inflammation, and angiogenesis. Cardiovasc. Res. 79, 581-588. PMID: 18550634.
73. Planat-Benard, V., Silvestre, J. S., Cousin, B., André, M., Nibbelink, M., Tamarat, R., Clergue, M., Manneville, C., Saillan-Barreau, C., Duriez, M., Tedgui, A., Levy, B., Pénicaud, L. and Casteilla, L. (2004) Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation 109, 656-663. PMID: 14734516.
74. The PSS Scale is reprinted with permission of the American Sociological Association, from Cohen, S., Kamarck, T., and Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24,* 386-396.
75. Cohen, S. and Williamson, G. Perceived Stress in a Probability Sample of the United States. Spacapan, S. and Oskamp, S. (Eds.) *The Social Psychology of Health.* Newbury Park, CA: Sage, 1988
76. Napadow V, Ahn A, Longhurst JC, et al. The status and future of acupuncture mechanism research. J Altern Complement Med. 2008;14(7):861–869. [PMC free article] [PubMed]
77. Al-Inany H. Acupuncture for infertility: a recently released evidence. Mid East Fertil Soc J. 2008;13(1):67.
78. Zheng CH, Huang GY, Zhang MM, Wang W. Effects of acupuncture on pregnancy rates in women undergoing in vitro fertilization: a systematic review and meta-analysis. Fertility and Sterility. 2012;97(3):599–611.
79. Kang HS, Jeong D, Kim DI, Lee MS. The use of acupuncture for managing gynaecologic conditions: An overview of systematic reviews. Maturitas. 2011;68(4):346–354.
80. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3124624/