Changes in serum cortisol and prolactin associated with acupuncture during controlled ovarian hyperstimulation in women undergoing in vitro fertilization–embryo transfer treatment

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Objective: To determine whether changes in serum cortisol (CORT) and PRL are affected by acupuncture (Ac) in Ac-treated IVF patients.

Design: Prospective cohort clinical study.

Setting: Private practice reproductive endocrinology and infertility clinic and private practice acupuncture consortium.

Patient(s): Sixty-seven reproductive-age infertile women undergoing IVF.

Intervention(s): Blood samples were obtained from all consenting new infertility patients and serum CORT and serum PRL were obtained prospectively. Patients were grouped as controls (IVF with no Ac) and treated (IVF with Ac) according to acupuncture protocols derived from randomized controlled trials.

Main Outcome Measure(s): Serum levels of CORT and PRL were measured and synchronized with medication stimulation days of the IVF cycle (e.g., day 2 of stimulation, day 3, etc.). Reproductive outcomes were collected according to Society for Assisted Reproductive Technology protocols, and results were compared between controls and those patients treated with Ac.

Result(s): CORT levels in Ac group were significantly higher on IVF medication days 7, 8, 9, 11, 12, and 13 compared with controls. PRL levels in the Ac group were significantly higher on IVF medication days 5, 6, 7, and 8 compared with controls.

Conclusion(s): In this study, there appears to be a beneficial regulation of CORT and PRL in the Ac group during the medication phase of the IVF treatment with a trend toward more normal fertile cycle dynamics. (Fertil Steril® 2009;92:1870–9. ©2009 by American Society for Reproductive Medicine.)

Key Words: IVF, acupuncture, cortisol, prolactin, pregnancy, traditional Chinese medicine, TCM

IVF represents the culmination of medical, scientific, and social evolution. It can be linked to over 2,000,000 babies worldwide and is the treatment of choice for over 1,000,000 infertile couples each year (1). If we track the improvements in reproductive outcomes (pregnancy rates) and or if we look at the percent of improvement in numbers of cycles of treatment in the United States, we observe a steady growth since data were first collected (1986). In 1996, Harlow et al. (2) presented their work showing a higher state of anxiety in women undergoing gonadotropin-stimulated IVF treatments and correlated it to levels of PRL and cortisol (CORT). In the same year, Stener-Victorin et al. (3) demonstrated positive effects of traditional Chinese medicine (TCM) in the form of acupuncture (Ac) on the pulsatility indices (PIs) of IVF patients. This was followed by Paulus et al. (4), whose pioneering work on the impact of Ac on reproductive outcomes of IVF patients has been verified by numerous large randomized controlled trials (RCTs) (5–8) and numerous cohort studies (9–15). Recently, Manheimer et al. (16) demonstrated, in a meta-analysis of the world's literature on Ac and IVF, a 10% improvement in reproductive outcomes when IVF patients added Ac to their treatment regimens.

When comparing these and other studies, the average improvement in pregnancy outcomes (ongoing or take home babies [THBs]) was 12%–14% for patients treated with IVF plus Ac (4–15). In the United States, to demonstrate a similar increase in reproductive outcomes with IVF alone, we would have to compare 1986 data with 1996 data (15% improvement) and 1996 data with 2003 data (8% improvement).

What remains a mystery is the biologic mechanism of the action of Ac on reproductive outcomes in patients treated with supraphysiologic levels of pharmaceuticals.

Recent studies have demonstrated how stress affects pregnancy rates. In one study by Gallinelli et al. (17), 40 infertile women were studied who were undergoing IVF-ET in a university hospital. Blood sampling was used. Gallinelli et al. correlated stress and immunity with human fertility. Women with functional chronic anovulation had higher serum CORT

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and cerebrospinal fluid corticotrophin-releasing hormone concentrations than healthy controls. This CORT hypersecretion has been reported in women undergoing IVF and ET who fail to achieve implantation. Moreover, a significant correlation between low adaptation to cognitive stress and poor outcomes has been reported in couples. Gallinelli et al. (17) concluded that a prolonged condition of stress caused a decreased ability to adapt; a transitory anxious state is associated with a high proportion of activated T cells in the peripheral blood, and such a condition reduces the embryo implantation rate. In another study, Smeenk et al. (18) examined urinary levels of stress hormones, adrenaline, noradrenalin, and CORT during treatment for self-reported stress to investigate the mechanism of the previously observed negative association for anxiety and depression with the outcomes of IVF/intracytoplasmic sperm injection. This was a prospective cohort study. Nocturnal urine samples were collected pretreatment, pre–oocyte retrieval, and before ET. Two questionnaires were administered to measure anxiety and depression. There was a significant positive correlation between urinary adrenaline concentrations at baseline and day of ET and the scores on depression at baseline. Women who had successful treatment had a lower concentration of adrenaline at oocyte retrieval and lower concentrations of adrenaline and noradrenalin at ET compared with the unsuccessful women. Smeenk et al. concluded that the association of adrenal hormone may be one of the links in the complex relationship between psychosocial stress and IVF outcomes. Klonoff-Cohen et al. (19) reported on the trauma associated with infertility. IVF contains a number of stressful aspects: daily injections, blood draws, ultrasound, laparoscopic surgery, and the possibility of failure at any of the various phases. He defined successful IVF as one gestational sac detected on the ultrasound. This cohort study consisted of 221 (151 completed the study) women undergoing gamete intrafallopian transfer (GIFT). Women completed two stress questionnaires, one at the first visit (baseline) and one at the time of their procedure. The baseline stressor was assessed by the positive and negative affect scale and bipolar profile of moods states; the investigators noted that there was a significant change in the perceived stress at baseline before and after hormone use. The women were categorized as having good levels of social support systems. Outcomes were interesting: for each unit increase in a woman's chronic negative-effect score on the stress survey, there was a 2% decrease in the number of oocytes retrieved. Similarly, when a woman's chronic negative-effect score was high, one to two fewer embryos were transferred. Stress and anxiety had an effect on successful pregnancies and live births. A 1-point increase in positive affect on the stress scale increased the live-birth delivery rate by 7 percent. Facchinetti et al. (20) demonstrated that an increased vulnerability to stress is associated with a poor outcome of IVF-ET treatment.

The purpose of this study was to investigate whether there are changes in the stress hormones (CORT and PRL) that are known to influence reproductive outcomes (i.e., pregnancy rates), in IVF patients treated with Ac. We also sought to determine in what direction the resultant stress hormones vary (more versus less).

MATERIALS AND METHODS

All patients seen at the Reproductive Medicine and Fertility Centers (RMFC) were invited to participate in our clinical trials by agreeing to have a vial of blood drawn during the normal standard times (7 A.M. to 9 A.M.). Thus, an extra vial of blood was collected on the following days: new patient visit, day 3 blood work, day of down-regulation, days of IVF medication for detection of IVF treatment effects (that is, stimulation days), day of hCG trigger, day 1 post-hCG trigger, and day of pregnancy detection. Each data point represents a normalized number standardized to start of stimulation. Demographic data on all aspects of patients and their partners as part of our routine computerized databases were collected, and these were used to derive demographic characteristics of patients in this study. Only those patients deemed eligible for IVF were included in this study. All patients completed IVF stimulation, egg retrieval, ET, and resultant pregnancy tests. Blood serum levels for CORT and PRL detection were by Immulite from DPC/Siemens (Princeton, NJ) (PRL intra-assay coefficient of variation [CV] = 6.8%; interassay CV = 9.6%; CORT intra-assay CV = 8.8%; inter-assay CV = 10%). Normal FSH (i.e., in female patients considered to have normal ovarian reserve) in our lab ranged from 2 to 10 mIU/mL.

Multiple variables can impact serum PRL and CORT levels (e.g., fasting state, medications, E_2 levels, etc.). To reduce these variables, the following steps were taken: all patients had serum levels checked before 8 A.M. as is the standard in our clinic and were not fasting; all patients received gonadotropins, baby aspirin, and GnRH agonist in the standard IVF protocol as stated; control and test patient demographics were consistent and statistically the same over 80 parameters as presented in prior publications (9–14).

This prospective cohort clinical trial of IVF patients was based on the following principals: [1] all new infertility patients signed informed consent forms (as part of their initial intake) to be part of a study that required a blood draw; [2] only lab personnel tracked participants; [3] neither the acupuncturist nor the medical staff knew which patients had agreed to be in the study; [4] all samples of blood were frozen for later analysis (analyses were done monthly to retain consistency of hormone levels); [5] the decision to have IVF was based solely on clinical evaluations; [6] the decision to have Ac was based solely on patient preference; [7] the statistician did not know who was in the study until after all bloods were collected, hormones analyzed, and IVF cycles completed before data analysis; [8] data were collected, stored, and then analyzed after all patients' birth outcomes were recorded, approximately 12 months from start of study; [9] data represent completed IVF cycle. The primary outcomes were detection of CORT and PRL serum levels at various stages of IVF

TABLE 1

Demographics of study patients and treatment cycle characteristics.

	Ac (n = 34)	Control (n = 33)
No. of prior IVF treatments	1.32 ± 0.4 (1–4)	1.44 ± 0.5 (1–4)
Age, years	34.6 ± 3.7 (25–41)	34.7 ± 3.6 (23–40)
Day3 FSH, IU/mL	10.2 ± 1.8 (4–12)	10.1 ± 1.8 (5–14)
BMI, kg/m^2	32.6 ± 8.5 (18–45)	33.2 ± 8.6 (22–48)
Weight, kg	67.3 ± 18.4 (48–95)	65.5 ± 18.1 (45–100)
E_2 on hCG day, pg/mL	3417 ± 104 (2201–5697)	3811 ± 153 (2315–6002)
P_4 on hCG day, ng/mL	1.51 ± .21 (1.2–3.9)	1.54 ± .24 (1.2–3.9)
Endometrial thickness on hCG day, mm	10.6 ± 1.1 (7.9–13)	9.4 ± 1.9 (8.1–14)
Oocytes retrieved	13.1 ± 2.2 (6–21)	12.6 + 2.0 (7–20)
Frozen embryos	5.4 ± 0.7 (0–9)	5.2 ± 0.7 (0–8)
Transferred embryos	2.8 ± 0.3 (2–5)	2.9 ± 0.3 (2–5)
Fertilization rate, %	79 ± 8 (66–94)	77 ± 9 (56–88)
Implantation rate, %	18.3	15.6

Note: Data are presented as mean ± SD (range). P = not significant (NS) for all comparisons (P>.05). All patients are IVF patients who underwent IVF medication stimulation, egg retrieval, and ET. N = 67.

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medication stimulation with comparisons between controls (IVF alone, n = 33) and treated patients (IVF plus Ac, n = 34). It is the trend in hormone secretions that are being compared. Each data point represents patients with that stimulation day blood draw. This does not assume that every patient has Ac on the same days of the stimulation cycles. The secondary study outcomes were pregnancy rates, miscarriage rates (spontaneous abortion), ectopic pregnancies, multiple pregnancy rates, and births per pregnancy, that is, take home babies (THBs). The study was not Institutional Review Board approved: all patients in our clinic are offered at the onset of their first visit the option to have their blood stored for research purposes; no additional blood was obtained from patients that was different from their normal course of care; patients consented, questions were answered, and patients had the option of not having their blood stored.

Treatment with Ac followed strict guidelines based on our previously published work (10–15) All acupuncturists who participated in our Acupuncture Consortium have a contractual obligation to provide Ac according to a strict protocol. This is not the usual method for TCM differentiation of syndromes treatment; however, to reduce treatment variability we conducted our study using only the following two modified protocols: Stener-Victorin (2) and Paulus (4); we define this unique combination as the Cridennda/Magarelli protocol. Patients are treated with the electrostimulation procedure (nine treatments) before egg retrieval and are treated with the pre-ET Ac within 24 hours before and 1 hour after ET. Data were collected and put into our computerized Ac database, and only those patients who met our strict Ac treatment criteria were included in this study. The strict criteria are defined as nine electrostimulation Ac treatments before egg retrieval and one pre- and one post-ET Ac treatment—for a total of 11 treatments (10–15).

Patients were treated at one center for IVF protocols, RMFC; however, the Ac was given by members of the contracted consortium (all of who were National Certification Commission for Acupuncture and Oriental Medicine [NCCAOM] certified and licensed acupuncturists) and occurred close to the residence of the patients. Most of the patients had already completed their IVF treatments with the subsequent blood tests for pregnancy before the data were made available for analysis.

For statistical analysis, t-test, χ^2, log rank analysis, and analysis of variance were used as appropriate. A power analysis was done based on a 26% difference in reproductive outcomes that was expected between the IVF and IVF + Ac group (based on our database of over 500 IVF cycles done over the last 5 years using the same Ac protocol). The analysis revealed that a minimum of 20 patients per arm of the study were needed.

RESULTS

Sixty-seven patients fulfilled the criteria for inclusion in the study. Table 1 demonstrates similar demographics including age, male and female fertility parameters, and embryology. No difference between FSH levels in the Ac and control groups were noted, but the average FSH for the study was elevated, suggesting decreased ovarian reserve, although this was not expressed by oocyte number, fertilization, or embryo development.

Reproductive outcomes are presented in Table 2, with noted statistically significant improvements when patients were treated with Ac for clinical pregnancies, miscarriage rates, THBs, and reduction in multiple pregnancies. These data are in keeping with our previously presented studies (10–15).

TABLE 2

Stress hormones: Reproductive outcomes data.

	Ac (n = 34)	Control (n = 33)	*P*
Pregnancy rate (+hCG)	18 (53)	14 (41)	<.05
Clinical pregnancy rate (+fetal heart beat [FHB]), %	51	37	<.05
Miscarriages	0 (0)	2 (6)	<.05
Ectopic pregnancies	1 (3)	3 (8)	NS
Birth per pregnancy	17 (94)	9 (64)	<.05
Multiple births	2 (11)	5 (33)	<.05

Note: Data are presented as n (%) unless otherwise specified. NS = not significant (*P*>.05). All patients are IVF patients who underwent IVF medication stimulation, egg retrieval, and ET. N = 67.

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TABLE 3

Impact of Ac on CORT levels during an IVF cycle.

Days from stimulation start	Ac (n = 34)	Controls (n = 33)	χ^2 Analysis
+2	14.1 ± 1.5	16.2 ± 1.7	NS
+3	16.2 ± 1.5	15.3 ± 1.4	NS
+4	13.9 ± 1.7	14.6 ± 1.9	NS
+5	12.0 ± 1.6	14.0 ± 1.6	NS
+6	13.9 ± 1.8	13.9 ± 1.7	NS
+7	16.2 ± 1.9	9.8 ± 1.2	<.05
+8	14.3 ± 1.4	9.7 ± 1.2	<.05
+9	14.1 ± 1.4	8.4 ± 1.1	<.05
+10	11.5 ± 1.2	7.8 ± 1.2	NS
+11	9.9 ± 1.3	4.3 ± 0.8	<.05
+12	8.4 ± 0.9	1.9 ± 0.2	<.01
+13	7.8 ± 1.0	1.8 ± 0.3	<.05
+14	3.0 ± 0.4	0	NS
+15	2.9 ± 0.4	0	NS

Note: Date are mean ± SD. Log rank comparison (a comparison of curves over time including all points) shows that there is a statistical difference between the curves (*P*<.05). Data represent the overall blood collection days for the IVF treatment cycle stimulation start + 2 days to ET. Gonadotropins were administered throughout the stimulation days until the day of hCG trigger that usually occurred at day 10 of the treatment cycle. (IVF timings vary from patient to patient, and the figure illustrates the typical timing of events.) Each data point represents a normalized number standardized to start of stimulation. Data represent the completed IVF cycle. All patients are IVF patients who underwent IVF medication stimulation, egg retrieval, and ET.

Magarelli. Changes in cortisol and PRL with acupuncture. Fertil Steril 2009.

When serum data were analyzed during the IVF cycles, there were variations in stimulation starts based on individual patient menstrual cycles. We standardized the data points collected by using the stimulation start date as day 1. All subsequent dates were relative to that date. Based on our observations (Tables 3 and 4), there were significant differences in CORT and PRL levels in the IVF-treated controls and Ac group (see Fig.1A and 1B)The PRL levels during the gonadotropins stimulation phase of an IVF treatment cycle rose above normal values then returned to normal values at the time of hCG administration (Fig. 1A, range 5–37 ng/mL; however, the follicular phase day 1–14 average was 12–17 ng/mL according to the Immulite ZPLKPR-1 information bulletin). CORT values remained in the normal range throughout the IVF treatment cycle studied (Fig. 1B, 5–25 ng/mL for morning values).

DISCUSSION

This study demonstrates biochemical differences in serum levels of CORT and PRL in patients receiving Ac along with their IVF treatments. We believe this is the first time a correlation between serum hormone levels and the use of Ac in an IVF cycle has been reported. We observed increased pregnancy rates in Ac-treated IVF patients and hypothesize that the increase in pregnancies is the result of the impact of Ac on PRL and CORT levels during the gonadotropins stimulation in the IVF treatment cycle.

The data regarding reproductive outcomes reported in this study are consistent with many RCTs and cohort studies that have been published since 1996 (4–15) and are consistent with a recently reported meta-analysis (16). This study supports one possible mechanism of action of Ac on IVF outcomes, that is, Ac induced biochemical changes in CORT and PRL during the gonadotropins stimulation in the IVF treatment cycle.

Many investigators have discussed the role of stress on reproductive failure (Nakumura et al. has a nice review; 21). There have been reports on how stress levels across stages of the IVF treatment cycle vary between pregnant and nonpregnant women (22). We suspected based on patient observations (Ac-treated patients "seemed" less stressed) and our own understanding of Ac's affects on the central nervous system (analgesia) that there would be changes (suppression) in these hormones. Our results demonstrated statistically significant changes; however, the results were different than we expected.

Osaki et al. (23) studied the relationship between PRL and prognosis for pregnancy in IVF-ET patients. Their study

TABLE 4

Impact of Ac on PRL levels during an IVF cycle.

Days from stimulation start	Ac (n = 34)	Controls (n = 33)	χ^2 Analysis
+2	13.3 ± 1.4	15.1 ± 1.9	NS
+3	16.3 ± 1.5	17.0 ± 2.3	NS
+4	19.4 ± 1.7	18.2 ± 2.0	NS
+5	26.0 ± 3.2	21.1 ± 2.5	<.05
+6	28.5 ± 4.6	21.2 ± 2.4	<.05
+7	34.9 ± 3.7	17.6 ± 2.1	<.001
+8	28.0 ± 3.2	16.9 ± 2,2	<.001
+9	19.4 ± 2.7	15.3 ± 1.7	NS
+10	14.6 ± 1.1	13.9 ± 1.4	NS
+14	9.7	10.9	NS

Note: Log rank comparison (a comparison of curves over time including all points) shows that there is a statistical difference between the curves ($P<.034$). Data represent the overall blood collection days for the IVF treatment cycle stimulation start to post-hCG day. Gonadotropins were administered throughout the stimulation days until the day of hCG trigger that usually occurred at day 10 of the treatment cycle. Few data points existed for post-hCG trigger, and the results are included to confirm trends only. (IVF timings vary from patient to patient, and the figure illustrates the typical timing of events.) Each data point represents a normalized number standardized to start of stimulation. Data represent the completed IVF cycle. All patients are IVF patients who underwent IVF medication stimulation, egg retrieval, and ET.

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specifically addressed midluteal PRL levels and found that lowered PRL levels at this point in an IVF cycle resulted in early pregnancy loss in those patients who became pregnant with IVF. This reduction in miscarriage rates is consistent with our studies that revealed a lower miscarriage rate in those patients treated with Ac (10, 11, 12, 14). In this study, Ac enhances PRL levels (above normal) before hCG trigger in an IVF treatment cycle (see Fig. 1A, PRL), and there were no differences in serum PRL levels after hCG trigger (see Fig. 1A, although few data points were available and the information was averaged).

A possible mechanism whereby PRL would improve IVF outcomes was postulated by Osaki et al. (23) They noted that PRL suppresses the immune response and that T-cell immunocompetence is maintained with PRL. They also noted that PRL is detected in the endometrium after day 23 of the menstrual cycle and that it increases with additional decidualization of the cavity, which roughly corresponds to the time at which implantation normally would occur. Could our "maintenance" of PRL levels in the Ac-treated IVF patients help in this process and produce better reproductive outcomes?

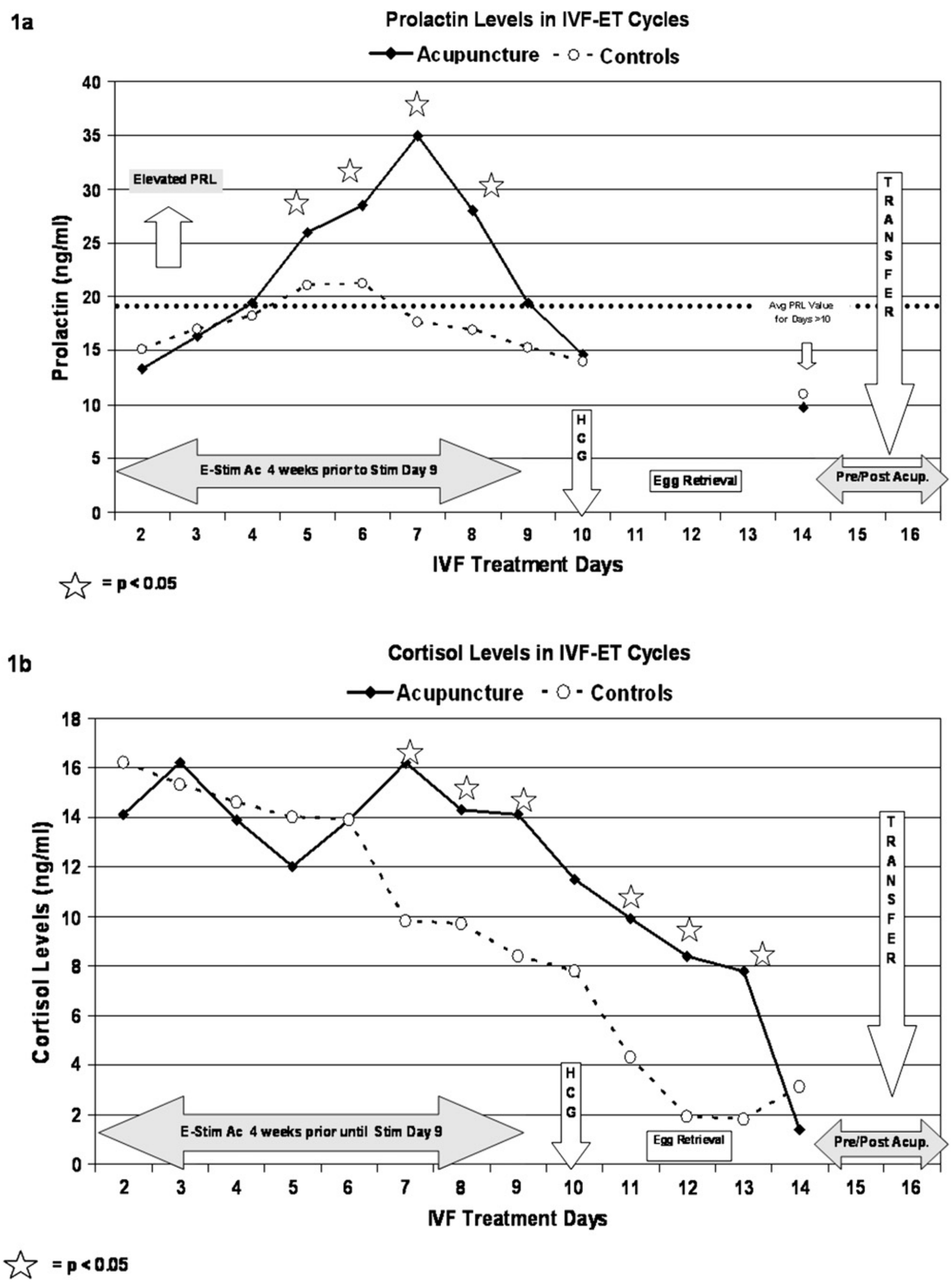
Merari et al. (24) addressed the issues of psychological and hormonal changes in the course of IVF. They believed both PRL and CORT were indicators of stress. They tested serum levels on day 3, day of retrieval, and day of ET. CORT levels were unchanged at these three points, and there were no differences in conception cycles versus nonconception cycles. In our data, on day 3 of stimulation and certainly on the day of ET there were similar, insignificant differences, in CORT levels. However, on the day of retrieval, there were significant elevations in CORT levels in the Ac group. It would be impossible to prove whether there were changes in CORT levels in the Merari et al. (24) study based on the data in their publication. We found significant improvements in IVF outcomes when patients were treated with Ac, but whether they were attributed to PRL or CORT levels is hard to decipher in this study. With regard to PRL, Merari et al. (24) found no differences at the time points measured. A slightly lower, not significant, reduction in PRL in the conception group was found in our study. This is in contrast to Osaki et al.'s data.

Merari et al. (24) also correlated psychological tests and hormonal levels and found that the CORT and PRL levels were significantly positively correlated in the conception cycles in women just before pregnancy tests. They found that the conception group was characterized by a negative significance for the states of anxiety and depression with both PRL and CORT. Merari et al. (24) suggested that PRL "might have served as an indicator of the stress level." In the nonconception cycles, the trend of no relationship between psychological measures and PRL and CORT levels was found and is reflected in our PRL data; no real changes in PRL in controls (non-Ac-treated groups) were noted in our study. However, there remains the observation that in our data, significantly lower levels of CORT were observed in controls.

Gonen and Casper (25) and Pattinson et al. (26) approached the role of PRL in IVF treatments by determining whether transient hyperprolactinemia (samples obtained before IVF treatments) had a negative effect on IVF parameters such as oocyte recovery, fertilization rates, and E_2 levels. They found that transient hyperprolactinemia had no negative effects (25, 26). Although not statistically significantly different, the number of ova retrieved and the fertilization rates were numerically higher in the transient hyperprolactinemic groups. The samples obtained in the Pattinson et al. study (26) were at the start of stimulation (stimulation day 1) and at the time of hCG. Three groups were created: PRL I had a PRL level on stimulation day 1 of <20 μg/L at the start with a rise over baseline of $\leq 200\%$; PRL II had a PRL level on stimulation day 1 of ≤ 20 with a rise over baseline of $>200\%$; and PRL III had a PRL level on stimulation day 1 of >20 at the start. The group that started with the higher PRL level had a 57% pregnancy rate compared with either of the other two groups: the pregnancy rate of PRL I was 25%, and of PRL II it was 21% (there were too few patients

FIGURE 1

Changes in serum levels of PRL (**A**) and CORT (**B**) associated with Ac (solid diamonds) in women undergoing COH for IVF-ET. Acupuncture was associated with a significant increase in PRL levels (solid diamonds) on stimulation days 4, 5, 6, and 7. On the other hand, CORT levels were significantly higher on stimulation days 7, 8, 9 and 11, 12, 13 in association with the Ac-treated group. All patients are IVF patients who underwent IVF medication stimulation, egg retrieval, and ET. Cridennda/Magarelli Ac protocol: electrostimulation Ac (nine treatments) occurred usually 4 weeks before the day of hCG. Pre-/post-Ac occurred within 24 hours before ET (one treatment) and 1 hour after ET (one treatment). (IVF timings vary from patient to patient, and the figure illustrates the typical timing of events.) Each data point represents a normalized number standardized to start of stimulation. Data represent the completed IVF cycle.



Magarelli. Changes in cortisol and PRL with acupuncture. Fertil Steril 2009.

to do a statistical analysis; the increase was about 28%–31%). In our study, those Ac-treated patients with PRL levels greater than those of controls during the stimulation phase of their IVF cycles had significantly higher pregnancy rates (in our most recent overview of over 576 IVF cycles, the overall improvement in IVF pregnancy rates were similar at 26%). It is interesting to note that in our study, the PRL levels observed in the Ac-treated patients after the start of stimulation and before the time of hCG trigger demonstrated no differences from controls; it was only during days 3–10 of the medications that significant differences arose. It is important to note that at the time of their IVF cycle, the patients would have had on average over nine Ac treatments. The authors believe that this suggests that there may be a cumulative effect of Ac on IVF outcomes and this may explain the consistency in improved reproductive outcomes in patients treated with the Cridennda/Magarelli protocol, consisting of 11 Ac treatments, nine before hCG plus pre and post ET. (10–15).

A curious aside, although there were very few participants in the Pattinson et al. study (26), those patients in the PRL III group (transient hyperprolactinemia group) had only singleton pregnancies, whereas the other two treatments groups both had multiple pregnancy rates of 31% and 50%, respectively (26). In our previously reported studies (13–15) and in the present study (Table 2), reductions in multiple pregnancies rates were noted in Ac-treated patients of up to 22% ($P<.05$).

In a murine model, levels of PRL and E_2 were found by Randall et al. (27) to correlate with improved polar body extrusion and overall improvements in oocyte maturity, fertilization, and embryo development. Mature follicles that produced fertilizable oocytes were found to contain higher follicular fluid PRL levels. In our Ac-treated patients, the serum levels of PRL just before hCG administration were statistically significantly higher (Table 4 and Fig. 1A). Although not a direct correlation to follicular levels, PRL is not formed in the follicles and would thereby require serum elevations to result in follicular increases. We are currently designing protocols to collect follicular fluids to confirm our hypothesis.

Stimulation protocols for IVF treatments have changed over the years. Historically, clomiphene citrate (CC) was used to stimulate controlled ovarian hyperstimulation (COH) in IVF cycles. Nilsson et al. (28) reported on the use of follicle size as a sole index of follicular maturity. In their study, they compared PRL levels in CC-treated patients (a now defunct method for COH for IVF) with hMG stimulation (this is the current standard treatment for IVF stimulation). In the CC-treated patients there were significantly diminished serum PRL levels at the time of hCG trigger compared with in the hMG-treated group. The average number of follicles developed and number of eggs retrieved demonstrated a twofold improvement in the hMG-treated patients for average number of follicles and a 137% increase for the number of eggs retrieved. Emmons and Patton (29) noted a similar response for poor responders when they were treated with Ac. Follicular fluid levels of PRL were numerically lower in the CC group compared with in the hMG group (28). Although this report more likely reflects the earliest stages in the development of IVF stimulation protocols, it is interesting to note that the PRL levels appeared to be suppressed and may have contributed to the overall poorer responses to CC versus hMG. Our data suggest that PRL level suppression may not be beneficial in IVF patients or conversely that elevations in PRL levels in the weeks just before egg retrieval may play a beneficial role.

Reinthaller et al. (30, 31) found an inverse correlation between follicular PRL levels and oocyte maturation. The more mature oocytes have lower levels of PRL and higher levels of P, T, and E_2 at the time of egg retrieval. Reinthaller et al. (30, 31) hypothesize that PRL inhibits the aromatization of androgens to estrogens in counterpoint to FSH stimulatory effects. PRL appears to exert a regulatory influence upon the estrogen-androgen metabolism in granulosa cells within the developing follicle, specifically on the aromatase enzyme. The recent advent of aromatase inhibitors for the treatment of infertility suggests that inhibition of aromatase during folliculogenesis may result in improved pregnancy outcomes in low-responder patients (32). In our first study comparing the impact of Ac on low responders, we found an over 50% improvement in THBs (live births) that was subsequently found to be numerically better in a review of over 576 patients in our database (10, and unpublished data). Could it be that the maintenance of PRL levels (intrafollicular) in our low-responder Ac-treated patients results in better outcomes via an aromatase inhibitor–like mechanism?

CORT in the form of an elevated CORT:hydrocortisone ratio in follicular fluid improves the rate of implantation leading to pregnancy (33). In our study, the Ac group demonstrated elevations of CORT from 4 days before hCG administration, and they remained higher than controls throughout the retrieval process (Fig. 1B, CORT), although the levels remained in the normal range for morning values. Poehl et al. (34) in a careful review of the need for psychotherapeutic counseling in IVF patients refers to reports that PRL and CORT correlate to higher stress levels in women who actually undergo the IVF stimulation versus those who receive oocyte donation. Although controversial, glucocorticoid adjuvant therapy for low-responder IVF patients has been reported by many. Ben-Rafael et al. (35) proposed that CORT simulates E_2 and P secretion by human granulosa cells and is an independent modulator different from the effects of FSH. Kemeter et al. (36) reported in 1986 that prednisolone improved IVF outcomes, while subsequent reviews noted no such positive effects (37). Bider et al. (38) reported that in animals, stimulation by corticosteroids is effective in facilitating the ovulatory responses. He suggested two modes of action: direct action of glucocorticoids on the ovaries and suppressive effects on the adrenal androgens. His study treated low responders with dexamethasone to determine whether there were improved outcomes. A low responder was defined as a patient who did not respond to CC and was 31–41 years of age. These criteria no longer would meet current definitions of a low responder for IVF. The results were less than spectacular in such a small study, and only two of the dexamethasone-treated patients became pregnant out of 20, versus one out of 22; compare this with 5% versus 4.5% versus 0% for the group before dexamethasone therapy (historic controls). This would appear to suggest that a glucocorticoid may improve outcomes. (At that time good responders had only a 20% chance of pregnancy with IVF; see www.cdc.gov.)

Although there does not appear to be an adequate body of knowledge to speculate on the actual impact of CORT and PRL on IVF outcomes based on changes during the menstrual cycle, we believe that the demonstrated changes in PRL and CORT (i.e., supraphysiologic levels of PRL during the gonadotropin phase of the IVF treatment cycle and significant changes in the CORT levels, still within the normal physiologic ranges) may be one mechanism of action for the effects demonstrated by our data and other studies in patients using Ac with IVF. There is a paucity of literature regarding CORT levels in IVF-ET treated patients. Keay et al. (33) was the only reference available that reflected a distant, not direct, correlation with our observations.

Any attempt to come up with a global explanation of the impact of Ac on IVF outcomes is premature. Our data would suggest that the nonphysiologic circumstances associated with COH with the added suppression of physiologic mechanisms for ovulation (E_2 triggering ovulation being suppressed by GnRH antagonists or agonist) may not reflect our "normal" view of how stress influences reproduction in the natural state. There are many publications suggesting a negative impact of stress on pulsatile GnRH secretion (39), but in all cases the patients (or animal models) were not subject to high doses of gonadotropins or to the suppressive effects of GnRH agonists or antagonists. This is the first time to our knowledge that Ac has been demonstrated to impact the hormones associated with reproduction in IVF patients. We also note that contrary to natural cycle conception in which pathologic elevations of PRL have been shown to decrease reproductive outcomes (39), we noted improved reproductive outcomes in Ac-treated COH cycle patients, and they were associated with elevated PRL during the gonadotropin phase of the IVF treatment cycles. The CORT data would seem to suggest that changes in the levels that start with the gonadotropins phase and continue into the post-hCG and retrieval phase results in improved reproductive outcomes in our Ac-treated group. This study only reports on our experience in COH IVF cycles.

For the TCM physician, stress plays a major role in our everyday existence. After years of trying to conceive, couples are at their wits' end and have been in the fight-or-flight mode for quite some time. Stress increases the CORT hormones as well as other neurochemicals. In TCM, stress is defined as liver Qi stagnation; this is characterized by anger, resentment, and unfulfilled desires. Physiologically, muscles become tight and blood vessels contract; the hyperactive sympathetic nervous system is in a constant state of hypervigilance with no mechanism to shut it off. Acupuncture may "correct" the negative effects of IVF medications on PRL as well as the adrenal response, and these effects may reduce stress as perceived by the patient.

There has been much controversy regarding the reported beneficial and potentially harmful impact of Ac on IVF outcomes (40). Some investigators believe (41, 42) that the number and acupoints as well as the timing and geographical location (travel to and from the ET location through city traffic) of the Ac treatments may explain the differences in outcomes observed (40). In our study, the Ac treatments were stringently regulated (by legal contract), and the timing of treatments was strictly monitored (again, by legal contract). This type of protocol is rather novel for Ac studies, and we believe the inclusion of these treatment controls plus the use of both electrostimulation and pre-/post-ET Ac treatments (Cridennda/Magarelli protocol) represents a unique and useful prototype for future research in this field. Our data suggest that patients receiving Ac weeks before the stimulation medications (usually 4 weeks at two treatments per week until day of retrieval) and in the times before egg retrieval and ET (as is the standard in our protocol, no patients receive electrostimulation Ac after egg retrieval) may benefit more than those patients treated just pre-/post-ET (i.e., the Paulus protocol; 4). We believe this to be true based on a preliminary review of those patients treated only with the pre-/postprotocol having no differences in reproductive outcomes compared with controls (unpublished data). In a recent review of over 576 IVF cycles in our database, 26% more pregnancies occurred when patients were treated with Ac and IVF (this is much greater than the percentage reported in the Manheimer meta-analysis; 16). Although direct comparisons with other Ac studies are not valid (owing to differences in protocols), our theory is that the differences and consistency of our reported pregnancy improvements (10–15) may be due to the cumulative affects of the treatments (nine electrostimulation, plus one pre- and one post-Ac treatment). The Cridennda/Magarelli protocol includes the Paulus protocol, and we believe that our data support that it may be the changes in the PRL levels during the gonadotropin phase of the IVF treatment (days 0–10) that most influence reproductive outcomes in patients treated with Ac (the Paulus protocol begins and ends around ET). This hypothesis also supports the addition of additional Ac treatments before ET since the PRL levels only differed during the gonadotropin phase of the IVF treatment cycle and returned to physiologic levels that were equivalent to those of the control patients at the time of hCG (see Fig. 1A). However, it is our theory that all Ac treatments in the Cridennda/Magarelli protocol work synergistically to significantly improve reproductive outcomes. The strength of Ac and TCM is that they restore balance or return the body to homeostasis. Our theory is that it may be that Ac neither increases or decreases hormone levels but simply returns the body to its natural state (we did note a significantly elevated PRL level in the Ac-treated group, however, above normal nonstimulated levels). The effects of Ac on PRL and CORT levels were noted even in the environment of extreme ovarian hyperstimulation as seen in typical IVF patients. More studies are needed to confirm these observations. We are currently collecting serum and follicular fluid samples to confirm the role of PRL and CORT as the mechanism of action of Ac on the observed improved reproductive outcomes. We believe our accumulated data represent the largest controlled trial measuring the impact of Ac on reproductive outcomes.

The mechanism of action for Ac's affects on IVF outcomes had been a mystery. With the data presented in this study, there does appear to be biochemical changes associated with the use of Ac in IVF that may explain the demonstrated improvements in reproductive outcomes. Acupuncture has been used for thousands of years, and while modern technology has assisted many couples to create families, we can expect even greater outcomes when both Eastern and Western medicines are combined. Although many factors were controlled for to reduce selection bias in our study, patients did choose to be treated with Ac; therefore, these controls may not eliminate selection bias or completely obviate a placebo effect. Larger RCT studies with additional rigor would help quantify these observations and are underway.

Our study demonstrates that Ac treatments (Cridennda/Magarelli protocol) improve reproductive outcomes in IVF patients. There is very little literature regarding the role of CORT and PRL in patients treated in controlled ovarian stimulation conditions such as IVF-ET. We believe that in addition to the understanding that our research brings to a potential mechanism of action of Ac in IVF-ET cycles, the physiologic implications of our observations may well add to our understanding of the conditions responsible for positive IVF outcomes.

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