GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(Published By: Global Institute for Research & Education)
www.gifre.org

Neuromuscular Electrical Stimulation and Biofeedback Therapy to Improve Endometrial Growth in Patients with thin Endometrium: A Randomized Controlled Study

Madafeitom Meheza Abide Bodombossou Djobo, Na Di, Xiaoli Chen, Zhuangyu He, Xiaomiao Zhao, Shaoqing Chen, Qingxue Zhang & Dongzi Yang[1]*

Department of Obstetrics and Gynecology, Reproductive Center, Memorial Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China
* Corresponding Author

Abstract

To evaluate the efficacy of noninvasive Neuromuscular electrical stimulation (NMES) in thin endometrium treatment, 115 patients (endometrium thickness ≤7mm) were recruited and randomly allocated to two groups. NMES group received NMES 3 to 4 times from day 9-10 and in comparison, a similar group received aspirin. Pre and post-treatment endometrium thickness, endometrial volume and Power Doppler Angiography (PDA) related parameters were measured by three-dimensional ultrasound. The endometrium was thicker in the NMES group compared with that in aspirin group [8.00 versus 7.72; P=0.028]. The endometrial and sub-endometrial volumes at final point also differ significantly between groups [2.58 versus 2.28; P=0.008 and 1.40 versus 1.21; P=0.001 for endometrial volume and sub-endometrial volume respectively. Endometrial flow index (FI), sub-endometrial vascularization index (sub-VI), sub-endometrial flow index (sub-FI) and sub-endometrial vascularization flow index (sub-VFI) differed statistically between groups (P=0.032, P=0.022, P=0.006 and P=0.018 respectively). In conclusion, NMES group showed better endometrial thickness, volume and vascularization than aspirin group.

Keywords: *Thin endometrium, neuromuscular electrical stimulation, endometrial vascularizationRCT Registration number: ChiCTR-TRC-11001787*

I. Introduction

In every day clinical practice, clinicians often see infertile patients with complex and unusual problem. One of the most challenging and frustrating problems to deal with is that of the patient with a poor endometrium or a thin uterine lining. Thin endometrium at the time of ovulation can be a concern and may be a factor in failure of implantation (Check, Nowroozi et al. 1991), poor placental development and miscarriage (Demirol and Gurgan 2004, Tan, Vandekerckhove et al. 2005). Normally, in response to estrogen, the uterine lining or endometrium grows about 1-2mm every other day. Thin endometrium more commonly occurs when the basal germinal endometrium, from which the full endometrial layer develops, is compromised in its response to estrogen by damage or reduced blood flow. Many studies have been reported that a poor uterine receptivity in women with thin endometrium is associated with the impairment of blood flow impedance through the endometrium(Hsieh, Tsai et al. 2000, Sher and Fisch 2002, Khairy, Banerjee et al. 2007). Adjuvant therapies are often used to improve endometrial development in women with thin lining during assisted reproductive technologies (ART)(Chen, Yang et al. 2006) (Hsieh, Tsai et al. 2000, Ledee-Bataille, Olivennes et al. 2002, Sher and Fisch 2002, Qublan, Amarin et al. 2008, Acharya, Yasmin et al. 2009, Dirckx, Cabri et al. 2009, Ho, Huang et al. 2009, Takasaki, Tamura et al. 2010, Gleicher, Vidali et al. 2011). In a pilot study published in 2011, we have shown that neuromuscular electrical stimulation (NMES) could be an effective option to manage women with thin endometrium (Bodombossou-Djobo, Zheng et al. 2011). NMES is the application of electrical current to the pelvic floor muscles. NMES combined with biofeedback may be useful in that the electrical stimulation provides a passive contraction that increases awareness of pelvic floor muscles contractions in general. Applying a low grade electrical current to pelvic floor muscles stimulates the pelvic muscles to contract. The purpose of this study was to investigate the effectiveness of the new therapy in a randomized controlled study and use three-dimensional ultrasound to objectively assess the response to therapy. The hypothesis was that by stimulating uterine smooth muscle to repeated contraction and relaxation, there will be an increased in muscle strength and blood supply towards the whole endometrial and the sub-endometrial regions that leads to peripheral tissue trophicity. By this hypothetical mechanism, NMES will likely correct the impairment of uterine blood flow and will increase the endometrial thickness.

II. Methods

The study was reviewed and approved by the Ethical Review Board of the Memorial Hospital of Sun Yat-Sen University. This was an open label, randomized controlled trial, interventional study, parallel assignment, comparing NMES with aspirin which was carried out from late December 2011 to May 2012 in Memorial Hospital of Sun Yat-Sen University. Pilot study publication (August 2011). After an observational study from September 2010 to April 2011, we were able to calculate a sample size. April 2011 to December 2011: sample size calculation after random table, decision of parallel treatment (Vitamin E, Estrogen or Aspirin), acquisition of three dimensional ultrasound equipment for objective assessment of endometrium in randomized control trial, screening for eligibility, concealment. Aspirin was retained after searches of medical literature reporting that Aspirin does not have a proliferative effect on endometrium.

The registration on December 2011. The patients' enrolment effectively started after registration, on late December (December 20th). Every patient gave a written informed consent prior to participating in the study (RCT Registration number: ChiCTR-TRC-11001787).

II.1 Study population

Women consulting for infertility at IVF center, Memorial Sun Yat-Sen Hospital of Sun Yat-Sen University were screening for eligibility, especially those undergoing timed intercourses on ovulation induction (OI, on Clomiphene citrate 50mg per day, from, D5 orally for 5 days or Letrozole 2.5 mg per day, from D5, orally for 5 days) or on natural cycle (NC, no fertility drug was used), intrauterine insemination (IUI) on OI or on NC and frozen-thawed embryo transfer (FET) on NC, OI cycle or on HRT(Estradiol valerate, Progynova; Bayer Schering Pharma, France was used for endometrial preparation, (see the pilot study for details on the endometrial preparation).

II.2 Inclusion and exclusion criteria

Women aged 20 through 39 years old were recruited into this study only if they were recorded at least 2 previous assisted reproductive treatment cycles failures in which the optimum endometrium thickness was ≤7 mm and signed the informed consent.

Women were excluded from participation if they have/were undergoing IVF/ICSI, a medical history that included pelvic cancer, severe endometriosis, Asherman's syndrome, sub-mucous myoma, adenomyosis, or congenital uterine anomalies by hysteroscopy, vaginitis, previous surgery in the pelvis, smokers and alcohol drinkers, neurologic disorders, systemic diseases, hypertension or diabetes mellitus, a use of an intrauterine device, and had previous use of long term hormonal contraception or other medication(s) which may have affected the uterus during the last 3 months or chronic use of any medication including nonsteroidal anti-inflammatory agents. Exclusion based on physical examination by the nurse practitioner (the physical therapist) occurred if women had vaginal wall prolapse, skin breakdown around the peri-anal region, rectal or vaginal bleeding, or absolute contraindications for pelvic neuromuscular electrical stimulation such as complete denervation of the pelvic floor (will not respond), dementia, cardiac pacemaker, unstable or serious cardiac arrhythmia, unstable seizure disorder, pelvic pain and swollen, painful hemorrhoids. Contraindications for aspirin such as women who were suffering from platelet dysfunction, thrombopenia, gastrointestinal ulcers, recurrent gastritis, aspirin hypersensitivity or who were on treatment with anticoagulants or aspirin were also considered for exclusion. We excluded women whose conditions warranted treatment with aspirin such as thrombophilia.

II.3 Study design

Screenings conformed to pre-assisted reproductive technologies (ART) protocols; hormones profile (day (D) 1-3 Serum PRL, FSH, LH, E2, and T) and pelvic ultrasound were completed.

II.4 Randomization

The sample size was calculated based on our pilot study. Randomization scheme has been constructed by using computer generated random numbers. Participants have been randomized to two study groups. Allocation to groups was by concealment. The concealments were made by a research assistant. A total sample size of 47 randomized subjects per treatment group was planned, with an additional 20 subjects (20%) to compensate for discontinued subjects. The enrollment of patients and follow-ups began on late December 2011.

II .5 Intervention

Group 1: NMES group underwent NMES therapy on D 9 or D10 for 3 to 4 times consecutively (qd for 20-30 minutes). PHENIX neuromuscular electrical stimulation therapy system USB 4 (Guangzhou Shanshan medical apparatus and instruments industry CO. LTD, Guangzhou, China) was used according to the manufacturer's recommended protocol for 20 to 30 minutes of intermittent vaginal electrical stimulation on the treatment days. The settings and steps of NMES have been detailed in our previous pilot study.

Group 2: Aspirin group received aspirin 100mg/day started from D 9 or D 10 until the day of urinary β-HCG test or serum β-HCG test.

Follow -up were done by ultrasound monitoring, using a 4D sonographic scanner (Voluson 730/ Voluson E8, GE Medical Systems, Kretz Ultrasound, Zipf, Austria) equipped with an automatic 6-12 MHz 4D probe, on D9-10, on the day of HCG injection (when lead follicles≥18mm or greater in diameter) or Serum E2≈200 pg/ml per follicle or D0, the start of progesterone supplementation for FET in the two groups), D16-18 to confirm ovulation, 2 weeks after HCG day for urine pregnancy test and up to 12 gestational weeks if pregnancy occurs.

II .6 Outcome Measures

Primary outcome measures assessed in this study were endometrial thickness and endometrial volume at the end of the treatment cycle.

Secondary outcome measures were endometrial and sub-endometrial vascularization by 3D PDA and the resistance index (RI), pulsatility index (PI) and eventually pregnancy rate.

II.7 Data analysis

Mean and standard deviation were used for description of variables which followed a normal distribution. Median and inter-quartile were used for description of variables which showed skew distribution and categorical variables were described as a percentage. Univariate analysis was performed with two sample t-tests for the continuous variables showing the normal distribution of data (Kolmogorov-Smirnov). The Mann–Whitney U-test was used for variables which were not normally distributed. For Categorical variables, statistical significances in frequency differences between groups were evaluated by using the chi-square test. Statistical analysis was performed with SPSS software, versions 16.0 (SPSS Inc., Chicago, IL, USA), with $P<0.05$ as the limit of significance.

III. Results

III.1 General characteristics of the study population

122 women were eligible to take part of the study. After exclusion and taking into account a dropout of +20% of included patients, we included 115 patients with 61 and 54 in NMES and aspirin group respectively. 12 of them (6 in the NMES group and 6 in the aspirin group) did not go through the treatment. In aspirin group, 3 women did not receive the treatment.1 woman was by random allocated to aspirin group, could not have the treatment because she has been taking the same treatment for 2 cycles of treatment and she required NMES therapy and conceived in that cycle (not included in the analysis). 2 refused to take part of the study after randomization and 3 did not come back for follow-up. In NMES group, 1 woman for lack of means, did not have NMES therapy, 2 did not start the treatment for unknown reasons. 2 were lost to follow-up on time due to the distance (from another hospital in cities close by).1 did not have a dominant follicle during monitoring and discontinued the intervention. A total of 55 and 48 women were randomized to NMES therapy and aspirin treatment groups respectively and effectively participated to the study and were further analyzed.

The general characteristics of the study subjects are shown in Table 1. The mean age of the study population was 30.74 ± 4.52 (range 21-39). The women in this study had mixed diagnosis. 51/103(50%) had primary infertility. 45 women out of 103 were diagnosed polycystic ovary syndrome (PCOS). 42 had tubal occlusion, and 71 with male factor. Their mean ages were 30.32 ± 4.57 and 31.23 ± 4.47 years for NMES and aspirin group respectively. Their mean body mass indexes (BMI) were 20.78 ± 3.24 and 21.82 ± 2.92 kg/m2 for NMES and aspirin respectively. The mean age, BMI, baseline hormones profile, endometrial thickness and endometrial volume at baseline (D 9-10) showed no significant difference between the two groups.

III.2 Primary outcome

After NMES therapy and aspirin treatment, the endometrium became thickened in the NMES group compared with that in aspirin group [8.00(6.66-9.22) versus 7.72(6.51-8.44); P=0.028] which was statistically significant. 32/55(58%) developed endometrial thickness equal to or more than 8 mm after NMES therapy in the NMES group and 16/48(33%) in aspirin group. The endometrial and sub-endometrial volume at final point also differ significantly between groups [2.58(1.90-3.45) versus 2.28(1.39-2.85); P=0.008 and 1.40(1.23-1.64) versus 1.21(0.92-1.44); P=0.001 for endometrial volume and sub-endometrial volume respectively] (Table 2).

III.3 Secondary outcome

The two groups did not differ significantly regarding endometrial vascularization index (VI) and vascularization flow index (VFI) at final point. But in another hand, endometrial flow index (FI), sub-endometrial vascularization index (sub-VI), sub-endometrial flow index (sub-FI) and sub-endometrial vascularization flow index (sub-VFI) differed statistically between NMES and aspirin groups (P=0.032, P=0.022, P=0.006 and P=0.018 respectively) (Table 3).

III.4 Pregnancy rate

Regarding the clinical pregnancy, there were 12 clinical pregnancies (12/53 or 23%) in the NMES group versus 7 (7/47 or 15%) in the aspirin group. This difference was not significant (P =0.445). the overall pregnancy rate was 19% (19/100). 2 women cancelled their cycles (1 in NMES and 1 in aspirin).

IV. Discussion

To our knowledge, this is the first randomized, open label controlled trial examining the effect of NMES compared to aspirin on endometrium thickness. Our main outcome of interest was the change in endometrial thickness and volume along with associated vascularization indices in the two groups. The data in this study have showed that NMES improved endometrial thickness, endometrial volume and endometrial vascularization in women with thin endometrium. NMES involves electrical stimulation of nerves and muscles with continuous short pulses of electrical curent (Kobetic, Triolo et al. 1997). NMES of the pudendal nerve (the nerve that innervates the pelvic floor muscles) at a relatively high frequency can cause a pelvic floor muscle contraction through a pudendal nerve reflex loop. The majority of the nerve fibers that supply the muscles of the bladder and pelvic operate at relatively high frequencies of 50 – 100 HZ. In this study, the frequency used was 40 Hz. Our choice of therapy frequency was based on the manufacturer's instructions while considering women ability to stand the therapy and achieve high levels of adherence (for more details on NMES protocol, confer to the pilot study).

During the last decade, the endometrium has been receiving increasing attention. A thin endometrial have been associated with a low pregnancy rates in many studies (Richter, Bugge et al. 2007, El-Toukhy, Coomarasamy et al. 2008, Miwa, Tamura et al. 2009). In our previous study (pilot study), we have shown that NMES could be an effective option to manage women with thin endometrium. It showed that pelvic floor NMES significantly enhanced endometrial thickness in patients with thin endometrium (Bodombossou-Djobo, Zheng et al. 2011). In the present study, we found that NMES have a positive effect on endometrial thickness, endometrial volume and endometrial and sub-endometrial vascularization. These findings are in agreement with the hypothesis we suggested in the earlier report. Decreases in voluntary muscle activation may negate increases in muscle size. Many studies have shown increased voluntary muscle activation with NMES (Gondin, Guette et al. 2005, Gabriel, Kamen et al. 2006). NMES-induced muscle contraction to generate sufficient muscle contraction for adequate exercise or functional purposes.

Aspirin is a salicylate drug, one of the most famous, cheapest, available and widely used drug in the world in patients with a wide range of therapeutic uses. Aspirin is often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication. Research on the use of aspirin during ART has yielded mixed results. Some studies suggest that aspirin therapy improves pregnancy rates (Weckstein, Jacobson et al. 1997, Rubinstein, Marazzi et al. 1999, Waldenstrom, Hellberg et al. 2004), others suggest it may increase the risk for miscarriage(Pakkila, Rasanen et al. 2005, Stern and Chamley 2006). Aspirin has been also proposed for the treatment of thin endometrium (Khairy, Banerjee et al. 2007) but seemed to be useless and inefficient from an evidence-based

medicine point of view(Poustie, Dodd et al. 2007, Siristatidis, Dodd et al. 2011). The theory of his mechanism of action is that aspirin improves blood flow inside the uterine lining and so forth. It has been establish that aspirin does not have any proliferative effect on endometrium and even more on increasing implantation rate and pregnancy rate (Dirckx, Cabri et al. 2009). The global idea concerning the endometrium development is that endometrium growth is dependent of its vascularity. Ng et al. have shown that endometrial and sub-endometrial vascularity measured by 3D power Doppler ultrasound was significantly lower (P <or= 0.003) in patients with low volume endometrium, but not in those with thin endometrium (Ng, Yeung et al. 2009). Merce et al. concluded that endometrial volume and 3D power Doppler indexes are statistically significant in predicting the cycle outcome (Merce, Barco et al. 2008). But this point needs several studies to be confirmed. A thin endometrium is also the actual hypothesis explaining the lower implantation rate in in vitro maturation (IVM) (Child, Gulekli et al. 2003, Holzer, Scharf et al. 2007). However the good question is, should we consider a thin endometrium rather than low endometrium volume. A low endometrial volume is defined by < 2.5 ml. In a clinical point of view, we needed to have some objective measurement to determine the probability of existing positive effect on endometrial thickness. Currently, although the standard measurement of endometrium thickness seems to be the measurement by standard two-dimensional ultrasound, we wanted to have some more objective measurements such as endometrial volume. As the new trend of 3-dimensionnal ultrasound offers new possibilities in endometrium exploration, we added endometrial volume measurement and associated parameters by three-dimensional Power Doppler ultrasound as secondary outcome. However, in this present study, aspirin did not improve endometrial thickness or volume or at least its effect was inferior to that seen in NMES group. The endometrial and sub-endometrial volumes at final point differed significantly between groups。These pinpoint out again a lack of evidence previously reported by many authors and two reviews in Cochrane Database for the routine use of low-dose aspirin in ART (Khairy, Banerjee et al. 2007, Poustie, Dodd et al. 2007, Dirckx, Cabri et al. 2009, Siristatidis, Dodd et al. 2011). Aspirin has an anti-platelet effect and causes a shift toward an increase in the synthesis of prostacyclin by its action on prostacyclin/thromboxane pathways, responsible for vasodilatation and an improved blood perfusion in many organs. The above-mentioned facts have led to the hypothesis that low-dose aspirin may improve uterine and ovarian perfusion and that aspirin might enhance endometrial receptivity and ovarian responsiveness as well (Rubinstein, Marazzi et al. 1999), which could result in better implantation and pregnancy rates after IVF or ICSI treatment. In this data, we did not attempt to compare the pregnancy rates between the two groups because of the heterogeneity in treatment protocols. Since our sample size was not chosen to analyze pregnancy rates, there could be a possible bias regarding the results. In a study by khairy et al, there was an improvement in uterine artery pulsatility index in patients taking low-dose aspirin (Khairy, Banerjee et al. 2007). However, it has been reported that the uterine arteries are not representatives of endometrium (Aytoz, Ubaldi et al. 1997, Salle, Bied-Damon et al. 1998). In this present study, instead of measuring uterine arteries pulsatility index and resistance index, we tried to measure the spiral arteries Doppler indices and our goal was to measure the vessels which are closer to the endometrium. But in some patients, we experienced the complete absence of blood flow color or very distant blood flow signals which made it impossible for us to have complete and uniform records about theses indices. From the data we obtained, there was no consistent decrease in impedance to uterine spiral arteries blood flow from baseline to final point in aspirin group patients. In contrast to these findings, there was a decrease in PI and RI values after NMES therapy in NMES group patients. This makes us believed that NMES not only improved endometrial thickness but also decreased PI and RI in patients with thin endometrium. Moreover, it has been shown that high blood flow impedance of uterine radial arteries and decreased in vascular endothelial growth factor (VEGF) expression are associated with poor endometrial growth(Senturk and Erel 2008, Takasaki, Tamura et al. 2010). A 2007 Cochrane Reviews and another one in 2008 found that women taking aspirin during IVF or ICSI were not significantly more likely to become pregnant than women in the controls groups (Duley, Henderson-Smart et al. 2007, Poustie, Dodd et al. 2007, Bromer, Cetinkaya et al. 2008). The findings of the updated 2011 Cochrane review mirror those of the 2007 review (Siristatidis, Dodd et al. 2011). Based on the available evidence, the authors have reached the same conclusion that no single outcome measure demonstrated a benefit with the use of aspirin, taken either preconceptually or at different stages of the treatment cycle (for example, during down-regulation, during stimulation of ovulation, after egg collection, or after confirmation of pregnancy by a pregnancy test or ultrasonography). There is no evidence that aspirin therapy during in IVF increases a woman's chances of becoming pregnant, according to an updated systematic review published in The Cochrane Library (Siristatidis, Dodd et al. 2011) . This meta-analysis combines the existing evidence and confirms that aspirin isn't likely to be of any major benefit in women undergoing IVF. This is a reason why aspirin was chosen and can be considered as a placebo in this present study.

In our study, aspirin was start on day 9-10 and it was discontinued as soon as the urinary β-hCG pregnancy test was done. There were no adverse reactions in both study groups; nevertheless, no serious adverse events such as gastrointestinal symptoms or blood loss regarding patients in aspirin group were reported. Possible factors accounting for the high adherence rate in the NMES group include the relative simplicity of the NMES training routine, the novelty of the modality and also that patients in our unit communicate between them, making participants to talk about the effect of the therapy. Once again, our study indicates the lack of evidence for the use of aspirin in patients undergoing ART with the aim of improving the endometrial thickness in patients with thin endometrium. We acknowledge that this study was sufficiently powered (power calculation of 80%) to show smaller differences regarding the potential benefits of NMES in women with thin endometrium.

V. Conclusion

Our randomized controlled trial showed better endometrial thickness, endometrial volume and vascularization in NMES group than that in aspirin group. There was a decrease in uterine spiral arteries Doppler indices in NMES group after NMES therapy compared to that in aspirin group. Despite this evidence, it is difficult to draw final conclusions about the exact mechanism of NMES effect on endometrium. However, more studies with greater sample size and with uniformity regarding treatment protocols are needed.

Grants

-Key project of research of National Ministry of Health (WGC [2010]439)
-The science technology research project of Guangdong Province (2012A030400010)

Conflict of interest

No conflict of interest

Acknowledgment

Special thanks to Chenqing Liang, Ultrasound Application Specialist from GE Healthcare, Sichuan Chengdu branch

VI. References

Acharya, S., E. Yasmin and A. H. Balen (2009). "The use of a combination of pentoxifylline and tocopherol in women with a thin endometrium undergoing assisted conception therapies--a report of 20 cases." Hum Fertil (Camb) **12**(4): 198-203.

Aytoz, A., F. Ubaldi, H. Tournaye, Z. P. Nagy, A. Van Steirteghem and P. Devroey (1997). "The predictive value of uterine artery blood flow measurements for uterine receptivity in an intracytoplasmic sperm injection program." Fertil Steril **68**(5): 935-937.

Bodombossou-Djobo, M. M., C. Zheng, S. Chen and D. Yang (2011). "Neuromuscular electrical stimulation and biofeedback therapy may improve endometrial growth for patients with thin endometrium during frozen-thawed embryo transfer: a preliminary report." Reprod Biol Endocrinol **9**: 122.

Bromer, J. G., M. B. Cetinkaya and A. Arici (2008). "Pretreatments before the induction of ovulation in assisted reproduction technologies: evidence-based medicine in 2007." Ann N Y Acad Sci **1127**: 31-40.

Check, J. H., K. Nowroozi, J. Choe and C. Dietterich (1991). "Influence of endometrial thickness and echo patterns on pregnancy rates during in vitro fertilization." Fertil Steril **56**(6): 1173-1175.

Chen, M. J., J. H. Yang, F. H. Peng, S. U. Chen, H. N. Ho and Y. S. Yang (2006). "Extended estrogen administration for women with thin endometrium in frozen-thawed in-vitro fertilization programs." J Assist Reprod Genet **23**(7-8): 337-342.

Child, T. J., B. Gulekli, C. Sylvestre and S. L. Tan (2003). "Ultrasonographic assessment of endometrial receptivity at embryo transfer in an in vitro maturation of oocyte program." Fertil Steril **79**(3): 656-658.

Demirol, A. and T. Gurgan (2004). "Effect of treatment of intrauterine pathologies with office hysteroscopy in patients with recurrent IVF failure." Reprod Biomed Online **8**(5): 590-594.

Dirckx, K., P. Cabri, A. Merien, L. Galajdova, J. Gerris, M. Dhont and P. De Sutter (2009). "Does low-dose aspirin improve pregnancy rate in IVF/ICSI? A randomized double-blind placebo controlled trial." Hum Reprod **24**(4): 856-860.

Duley, L., D. J. Henderson-Smart, S. Meher and J. F. King (2007). "Antiplatelet agents for preventing pre-eclampsia and its complications." Cochrane Database Syst Rev(2): CD004659.

El-Toukhy, T., A. Coomarasamy, M. Khairy, K. Sunkara, P. Seed, Y. Khalaf and P. Braude (2008). "The relationship between endometrial thickness and outcome of medicated frozen embryo replacement cycles." Fertil Steril **89**(4): 832-839.

Gabriel, D. A., G. Kamen and G. Frost (2006). "Neural adaptations to resistive exercise: mechanisms and recommendations for training practices." Sports Med **36**(2): 133-149.

Gleicher, N., A. Vidali and D. H. Barad (2011). "Successful treatment of unresponsive thin endometrium." Fertil Steril **95**(6): 2123 e2113-2127.

Gondin, J., M. Guette, Y. Ballay and A. Martin (2005). "Electromyostimulation training effects on neural drive and muscle architecture." Med Sci Sports Exerc **37**(8): 1291-1299.

Ho, M., L. C. Huang, Y. Y. Chang, H. Y. Chen, W. C. Chang, T. C. Yang and H. D. Tsai (2009). "Electroacupuncture reduces uterine artery blood flow impedance in infertile women." Taiwan J Obstet Gynecol **48**(2): 148-151.

Holzer, H., E. Scharf, R. C. Chian, E. Demirtas, W. Buckett and S. L. Tan (2007). "In vitro maturation of oocytes collected from unstimulated ovaries for oocyte donation." Fertil Steril **88**(1): 62-67.

Hsieh, Y. Y., H. D. Tsai, C. C. Chang, H. Y. Lo and C. L. Chen (2000). "Low-dose aspirin for infertile women with thin endometrium receiving intrauterine insemination: a prospective, randomized study." J Assist Reprod Genet **17**(3): 174-177.

Khairy, M., K. Banerjee, T. El-Toukhy, A. Coomarasamy and Y. Khalaf (2007). "Aspirin in women undergoing in vitro fertilization treatment: a systematic review and meta-analysis." Fertil Steril **88**(4): 822-831.

Kobetic, R., R. J. Triolo and E. B. Marsolais (1997). "Muscle selection and walking performance of multichannel FES systems for ambulation in paraplegia." IEEE Trans Rehabil Eng **5**(1): 23-29.

Ledee-Bataille, N., F. Olivennes, J. L. Lefaix, G. Chaouat, R. Frydman and S. Delanian (2002). "Combined treatment by pentoxifylline and tocopherol for recipient women with a thin endometrium enrolled in an oocyte donation programme." Hum Reprod **17**(5): 1249-1253.

Merce, L. T., M. J. Barco, S. Bau and J. Troyano (2008). "Are endometrial parameters by three-dimensional ultrasound and power Doppler angiography related to in vitro fertilization/embryo transfer outcome?" Fertil Steril **89**(1): 111-117.

Miwa, I., H. Tamura, A. Takasaki, Y. Yamagata, K. Shimamura and N. Sugino (2009). "Pathophysiologic features of "thin" endometrium." Fertil Steril **91**(4): 998-1004.

Ng, E. H., W. S. Yeung and P. C. Ho (2009). "Endometrial and subendometrial vascularity are significantly lower in patients with endometrial volume 2.5 ml or less." Reprod Biomed Online **18**(2): 262-268.

Pakkila, M., J. Rasanen, S. Heinonen, H. Tinkanen, L. Tuomivaara, K. Makikallio, M. Hippelainen, J. S. Tapanainen and H. Martikainen (2005). "Low-dose aspirin does not improve ovarian responsiveness or pregnancy rate in IVF and ICSI patients: a randomized, placebo-controlled double-blind study." Hum Reprod **20**(8): 2211-2214.

Poustie, V. J., S. Dodd and A. J. Drakeley (2007). "Low-dose aspirin for in vitro fertilisation." Cochrane Database Syst Rev(4): CD004832.

Qublan, H., Z. Amarin, M. Al-Qudah, F. Diab, M. Nawasreh, S. Malkawi and M. Balawneh (2008). "Luteal phase support with GnRH-a improves implantation and pregnancy rates in IVF cycles with endometrium of <or=7 mm on day of egg retrieval." Hum Fertil (Camb) **11**(1): 43-47.

Richter, K. S., K. R. Bugge, J. G. Bromer and M. J. Levy (2007). "Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of in vitro fertilization with transfer of two blastocyst-stage embryos." Fertil Steril **87**(1): 53-59.

Rubinstein, M., A. Marazzi and E. Polak de Fried (1999). "Low-dose aspirin treatment improves ovarian responsiveness, uterine and ovarian blood flow velocity, implantation, and pregnancy rates in patients undergoing in vitro fertilization: a prospective, randomized, double-blind placebo-controlled assay." Fertil Steril **71**(5): 825-829.

Salle, B., V. Bied-Damon, M. Benchaib, S. Desperes, P. Gaucherand and R. C. Rudigoz (1998). "Preliminary report of an ultrasonography and colour Doppler uterine score to predict uterine receptivity in an in-vitro fertilization programme." Hum Reprod **13**(6): 1669-1673.

Senturk, L. M. and C. T. Erel (2008). "Thin endometrium in assisted reproductive technology." Curr Opin Obstet Gynecol **20**(3): 221-228.

Sher, G. and J. D. Fisch (2002). "Effect of vaginal sildenafil on the outcome of in vitro fertilization (IVF) after multiple IVF failures attributed to poor endometrial development." Fertil Steril **78**(5): 1073-1076.

Siristatidis, C. S., S. R. Dodd and A. J. Drakeley (2011). "Aspirin for in vitro fertilisation." Cochrane Database Syst Rev(8): CD004832.

Stern, C. and L. Chamley (2006). "Antiphospholipid antibodies and coagulation defects in women with implantation failure after IVF and recurrent miscarriage." Reprod Biomed Online **13**(1): 29-37.

Takasaki, A., H. Tamura, I. Miwa, T. Taketani, K. Shimamura and N. Sugino (2010). "Endometrial growth and uterine blood flow: a pilot study for improving endometrial thickness in the patients with a thin endometrium." Fertil Steril **93**(6): 1851-1858.

Tan, B. K., P. Vandekerckhove, R. Kennedy and S. D. Keay (2005). "Investigation and current management of recurrent IVF treatment failure in the UK." BJOG **112**(6): 773-780.

Waldenstrom, U., D. Hellberg and S. Nilsson (2004). "Low-dose aspirin in a short regimen as standard treatment in in vitro fertilization: a randomized, prospective study." Fertil Steril **81**(6): 1560-1564.

Weckstein, L. N., A. Jacobson, D. Galen, K. Hampton and J. Hammel (1997). "Low-dose aspirin for oocyte donation recipients with a thin endometrium: prospective, randomized study." Fertil Steril **68**(5): 927-930.

Annexure:

Table 1 General characteristic of the two groups (NMES & Aspirin)

Variables	NMES group (N=55)	Aspirin group(N=48)	P value
Age (years)	30.32 ± 4.57	31.23 ± 4.47	0.288
BMI (kg/m^2)	20.78 ± 3.24	21.82 ± 2.92	0.095
bFSH (IU/ml)	7.02 ± 1.93	8.50 ± 9.47	0.289
bLH (IU/ml)	5.89 ± 3.26	5.77 ± 3.68	0.851
bE2(ng/l)	50.14 ± 37.89	50.91 ± 42.04	0.922
bT (mmol/ml)	1.12(0.86-1.7)	1.35(0.73-2.00)	0.722
Infertility			
Primary	28(50%)	23(48%)	
Tubal factor	24(41%)	18(38%)	
Male factor	38(64%)	33(69%)	
PCOS	24(41%)	21(45%)	
Abortion history	19(33%)	15(31%)	

Data are presented as number (percentage) N (%), mean ± SD, or median (inter-quartile range) and analyzed by Student t-test, Non-parametric test or Chi-square as appropriate. P< 0.05 was significant. bFSH: baseline Follicle Stimulating Hormone, bE_2: baseline estradiol, bT: baseline Testosterone, BMI: Body Mass Index, PCOS: Polycystic Ovary Syndrome, NMES: Neuromuscular Electrical Stimulation.

Table 2 Comparison of endometrial thickness, endometrial volume and sub-endometrial volume and pregnancy rate between the two groups

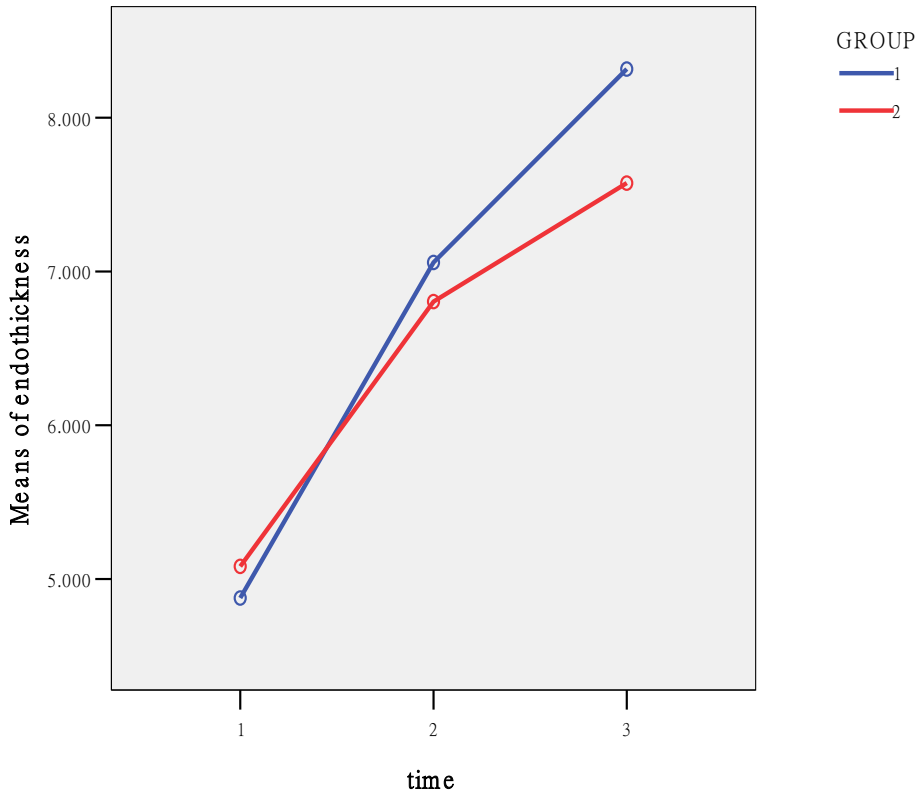
Variables	NMES group(N=55)	Aspirin group(N=48)	P value
Endometrial thickness at baseline(mm)	4.79(3.89-5.68)	4.96(4.06-5.89)	0.237
Endometrial thickness between the 2 points	7.14(6.13-8.13)	6.89(5.49-7.77)	0.384
Endometrial thickness at hCG day(mm)	8.00(6.66-9.22)	7.72(6.51-8.44)	0.028
Endometrial volume at baseline(cm^3)	1.08(0.74-1.71)	1.21(0.73-1.60)	0.833
Endometrial volume at hCG day(cm^3)	2.58(1.90-3.45)	2.28(1.39-2.85)	0.008
Sub-endometrial volume at baseline(cm^3)	0.85(0.70-1.16)	0.85(0.61-1.13)	0.968
Sub-endometrial volume at hCG (cm^3)	1.40(1.23-1.64)	1.21(0.92-1.44)	0.001
Pregnancy rate	12/53(23%)	7/47(15%)	0.445

Data are presented as median (inter-quartile range) and analyzed by Non-parametric test. P< 0.05 was significant. Between the 2 points: time between baseline point and hCG day point. P value was adjusted by Bonferonni correction.

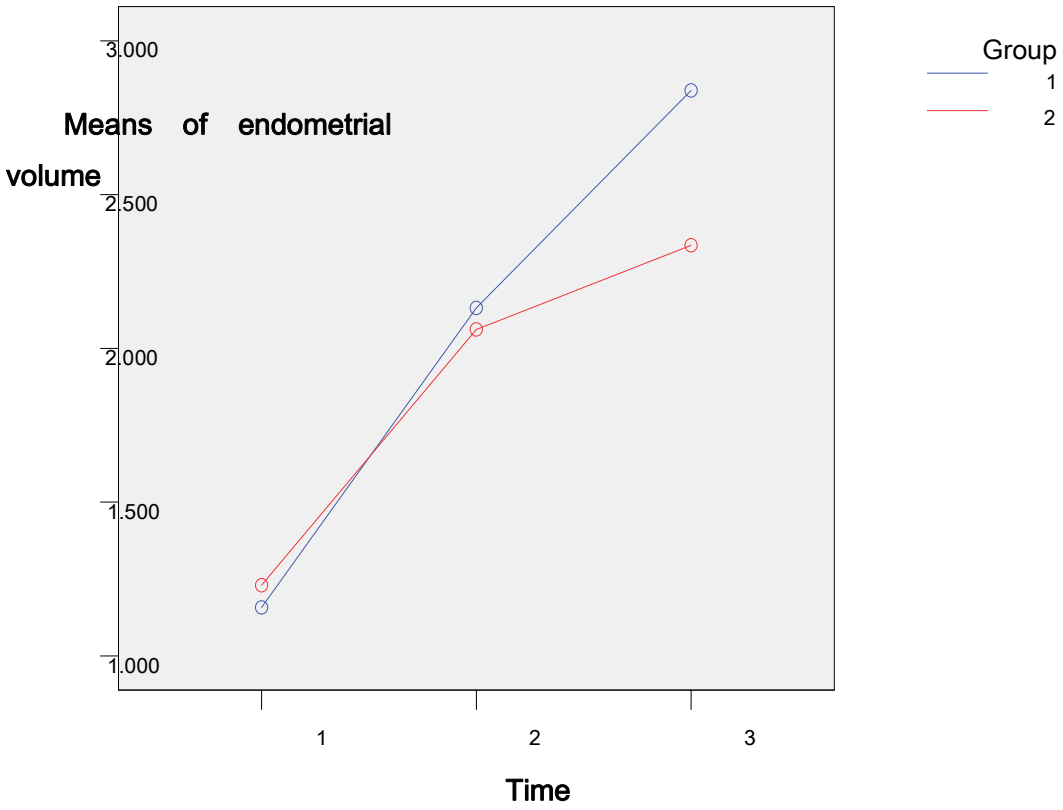
Table 3 Comparison of endometrial and sub-endometrial angiographic indices between the two groups

Variables	NMES group(N=55)	Aspirin group(N=48)	P value
Endometrial vascularization			
1Vascularization Index	1.11(0.32-4.65)	1.28(0.28-3.73)	0.663
3 Vascularization Index	3.47(1.69-5.71)	2.39(0.93-5.38)	0.068
1 Flow Index	25.70(22.31-27.95)	23.52(21.19-26.38)	0.058
3 Flow Index	25.75(24.08-28.37)	24.37(23.16-27.10)	0.032
1 Vascularization Flow Index	0.27(0.07-1.32)	0.29(0.06-1.07)	0.666
3 Vascularization Flow Index	0.96(0.43-1.50)	0.52(0.22-1.48)	0.056
Sub-endometrial vascularization			
1 Sub-Vascularization Index	4.01(1.39-11.40)	2.09(1.28-7.51)	0.366
3 Sub-Vascularization Index	8.68(4.82-15.66)	5.92(1.55-11.84)	0.022
1 Sub-Flow Index	26.48(24.15-29.38)	23.89(22.21-26.99)	0.003
3 Sub-Flow Index	27.85(25.38-29.45)	26.22(23.81-28.31)	0.006
1 Sub-Vascularization Flow Index	1.11(0.36-3.19)	0.79(0.33-2.11)	0.42
3 Sub-Vascularization Flow Index	2.26(1.22-4.36)	1.57(0.39-3.26)	0.018
Doppler indices			
1Pulsatility Index(PI)	1.14(0.96-1.47)	1.00(0.81-1.27)	0.009
3Pulsatility Index(PI)	1.11(0.87-1.24)	1.00(0.82-1.16)	0.202
1 Resistance Index(RI)	0.65(0.59-0.75)	0.61(0.54-0.72)	0.290
3 Resistance Index(RI)	0.61(0.57-0.69)	0.61(0.55-0.64)	0.292

Data are presented as median (inter-quartile range) and analyzed by Non-parametric test. P< 0.05 was significant. 1: baseline (D8-10), 2: time between 1 and 2, 3: hCG administration day=final point.



Graph 1 Change of endometrial thickness means over time in the two groups.
1: baseline (D8-10), 2: time between 1 and 2, 3: hCG administration day=final point. Group1=NMES Group;Group2=Aspirin group



Graph 2 Change of endometrial volume means over time in the two groups

1: baseline (D8-10), 2: time between 1 and 2, 3: hCG administration day=final point. Group1=NMES Group;Group2=Aspirin group