

Tadalafil for Endometrial Growth in Clomiphene Citrate stimulated cycles in an IUI programme: A pilot study

D.H. MENDEZ LOZANO^{1,2}, M.V. LENERO¹, R.L. GONZALEZ^{1,2}, J.B. SCHEFFER³, M.T. GONZALEZ, Y. BARRON¹, R. FRYDMAN⁴

¹School of Medicine, Tecnológico de Monterrey, Ave. Morones Prieto No. 3000 Pte, Col. Los Doctores, 64710, Monterrey, Nuevo León, México.

²Center for Reproductive Medicine CREASIS San Pedro, Monterrey, México.

³IBRRA – Instituto Brasileiro de Reprodução Assistida, Belo Horizonte, MG, Brazil.

⁴Département d'Obstetrics et Gynecology, Hôpital Foch de Suresnes, 40 rue Worth, Suresnes, France.

Correspondence at: danielmendez@itesm.mx

Abstract

Aim of the study: The objective of this study was to assess the impact of tadalafil on endometrial growth, the uterine artery pulsatility index (PI) and the uterine artery resistance index (RI) in patients under clomiphene ovarian stimulation for intrauterine insemination (IUI).

Methods: This randomized crossover study included 30 patients with a normal endometrium over 53 cycles, and 46 of those cycles in 23 patients were included in the analysis. In group A the patients were under 100 mg clomiphene daily for five days (2-6) and 5 mg tadalafil daily for 7 days (4-10). For Group B (control) the patients only received clomiphene. Measurements of the endometrium, PI, RI and estradiol determinations were taken on cycle days 4, 8 and 10.

Results: We observed a better endometrial growth in Group A compared to Group B: 7.5 ± 2.1 mm vs 5.5 ± 1.2 mm, $P < 0.0002$ and 8.9 ± 1.8 mm vs 6.3 ± 1.8 mm, $P < 0.0002$ on days 8 and 10, respectively. Additionally, a progressive decrease in the RI was observed in Group A but not in Group B from day 8 (0.77 ± 0.15 vs 0.85 ± 0.18 , $P = 0.059$) to day 10 (0.74 ± 0.20 vs 0.87 ± 0.14 , $P < 0.017$). However, no differences were observed in PI or serum estradiol between Group A and Group B.

Conclusion: the use of tadalafil improved endometrial growth in patients under clomiphene ovarian stimulation with no significant effect on the uterine artery Pulsatility Index and serum estradiol.

Key words: Tadalafil, Endometrial growth, Pulsatility Index, Resistance Index, Clomiphene citrate, ovarian stimulation.

Introduction

Ovarian stimulation with clomiphene citrate for intrauterine insemination (IUI) has been discussed because of its negative effect on endometrial development (Kafy and Tulandi, 2007; Jirge and Patil, 2010; Reynolds et al., 2010; Palatnik et al., 2012). Moreover, better endometrial growth has been associated with a higher pregnancy rate (Abdalla et al., 1994; Dessolle et al., 2009; Palatnik et al., 2012). Thus, several efforts have been made to increase endometrial development with variable and contradictory results (Senturk and Erel, 2008;

Takasaki et al., 2010; Gleicher et al., 2013; El Refaey et al., 2014; Lebovitz and Orvieto, 2014).

Short-acting inhibitors of phosphodiesterase 5 (PDE5), such as sildenafil, have been proposed as useful agents for increasing endometrial development in patients with an endometrium that had previously undergone surgical treatment. The proposed principal mechanism of these drugs for obtaining a better endometrium is supposed to be an improvement of vascular flow (Sher and Fisch, 2000; Paulus et al., 2002; Sher and Fisch, 2002; Zinger et al., 2006; Dehghani Firouzabadi et al., 2013).

However, the possible utility of administering a longer-acting PDE5 inhibitor, such as tadalafil, to achieve a thicker endometrium in patients with a non-operated endometrium under clomiphene ovarian stimulation remains unknown (Forgue et al., 2006; Wrishko et al., 2009).

Therefore, the principal aim of the present study was to assess the potential usefulness of tadalafil in terms of improving endometrial development in patients under clomiphene ovarian stimulation. A cross-over randomized study design was used and the outcome measures were endometrial thickness, the uterine artery pulsatility index, the uterine artery resistance index and serum estradiol.

Materials and Methods

Patients

We prospectively studied 30 infertile female patients during 53 cycles in an IUI programme using clomiphene citrate (CC) ovarian stimulation. Patients 18-42 years of age attending the Center of Reproductive Medicine CREASIS and Centro Médico Zambrano Hellion at Tecnológico de Monterrey University were invited to participate and recruited from September 2014 to August 2015. Patients were randomized at the beginning of this cross-over study to start in either Group A (CC + tadalafil) or Group B (only CC), and because pregnancy was achieved in the first cycle for 7 patients, we included the other 23 patients who completed both cycles (46 cycles) in the final analysis. They were followed up until clinical pregnancy could be confirmed.

Informed written consent was obtained from all patients and this investigation received the approval of our internal Institutional Review Board on 27 August 2013 (reference no. IPDE5EC). The informed consents of participants were recorded in individual files. The trial was registered as ISRCTN95746843.

The inclusion criteria were as follows: (i) no previous endometrium surgery (ii) no history of smoking (iii) both ovaries present and without morphological abnormalities (iv) regular menstrual cycles of 27 to 32 days in duration (v) no current or past diseases affecting the ovaries or gonadotrophin or sex steroid secretion, clearance or excretion (vi) no arterial hypertension (vii) body mass index (BMI) ranging between 18 and 25 kg/m² (viii) no current hormone therapy and (ix) adequate visualization of both ovaries and the endometrium on transvaginal ultrasound scan.

Protocol

Patients were randomized using previously sealed envelopes to start in either Group A or Group B. Only the patients who completed both cycles were included in the final analysis of this crossover study. For Group A patients were administered 100 mg/day clomiphene citrate from the 2nd to the 6th day of the cycle and 5 mg/day tadalafil from the 4th day to the 10th day of the cycle. Both drugs were administered at night. For Group B patients only received 100 mg/day clomiphene citrate from the 2nd to the 6th day of the cycle. All patients were evaluated on days 4, 8 and 10 of both cycles with ultrasonographic and hormonal measurements.

Ultrasound scans were performed with a vaginal probe (Logiq 3 Pro; General Electric Medical Systems, transvaginal probe E8C of 4-10 Mhz). On sagittal view of the uterus, the endometrial thickness was measured. Using colour doppler the uterine artery pulsatility index (PI) and the uterine artery resistance index (RI) were measured, and the means for both sides were calculated. To measure the uterine artery indices, the insonation angle used was between 0° and 30°, and the waveforms in three to six cardiac cycles were obtained from the ascending main branch of the uterine artery at the cervix level before it entered the uterus in a longitudinal plane. Additionally all data acquisitions were obtained under the same Doppler settings: gain 30, PRF 1.5 kHz and frequency 4.0 MHz. The follicle measurements were the means of two orthogonal diameter measurements and were obtained using the tissue harmonic system, as previously described (Fanchin et al., 2003; 2005, 2007; Mendez Lozano et al., 2008). One blind single operator performed all measurements. Additionally, all data were digitally stored and were not analysed until the end of the study.

Hormonal measurements of serum estradiol, LH and progesterone were also performed on days 4, 8 and 10 of the cycles. All samples were obtained at 8 AM in the morning at the CREASIS (Center for Reproductive Medicine). The serum estradiol concentrations were determined using an automated multi-analysis system and a chemiluminescence technique (Advia-Centaur, Bayer Diagnostics, Puteaux, France). For estradiol, the lower detection limit was 15 pg/ml, the linearity was up to 1,000 pg/ml and the intra- and inter-assay coefficients of variation were 8 and 9%, respectively.

Finally, the women received a 10 000 IU hCG injection intramuscularly when the dominant follicle diameter exceeded 18 mm and the intrauterine insemination was performed 36 hours after the injection.

Statistical analysis

The measures of central tendency and variability used when the data distribution was normal were the mean and standard error of the mean and those used when normality could not be ascertained were the median and range respectively. Data distribution normality was assessed by the Kolmogorov Smirnov test. Unpaired data were compared by the unpaired Student's t-test or the Mann Whitney test, as appropriate. Paired data were compared by the Wilcoxon signed rank test. The chi-squared and Fisher's exact tests were used to compare categorical variables. A P value < 0.05 was considered to indicate a statistically significant difference. Even when this is the first study with tadalafil, we calculated the sample size according to differences observed on endometrium grow with other PDE5 inhibitors. Thus, we calculate 18 patients needed in this crossover study for a difference of at least 1 mm on endometrial thickness observed in 30% of them with a 0.05% significance level and 0.8 of power. In this crossover study, only patients with their own control cycle were considered for analysis.

Results

Thirty participants were included in this cross-over randomized study (Fig. 1). Fifty-three cycles of ovarian stimulation and intrauterine insemination were included. Those 53 cycles included 26 cycles with clomiphene and tadalafil treatment (Group A) and 27 cycles with clomiphene citrate treatment alone (Group B). As expected, 23 patients underwent both cycles, as pregnancy was achieved during the first cycle in 7 patients. Thus, 46 cycles were included in the analysis of the variables of interest. During the ovarian stimulation cycles, no additional drugs were administered and endometrial ovarian stimulation was never performed out of schedule, regardless of the response to the treatment.

Regarding patient demographics, the average age of our patients was 31.8 ± 0.9 years, the basal FSH level was 4.9 ± 0.4 IU/ml, the antral follicle count (AFC) was 16.7 ± 1.6 follicles and the total motile spermatozoa in the initial semen sample was 14.3 ± 2 million. The most frequent causes of infertility were male factor (65%), unexplained (20%) and tubal abnormalities (15%). Only patients who completed both cycles were included in the paired analysis and hypothesis testing. Therefore, for 23 patients, the two cycles were compared to determine differences in endometrial thickness, pulsatility index and resistance index on days 4, 8 and 10 of the cycle.

On day 4 of the cycle, we found no differences between Group A and B in the endometrial thickness (4.5 ± 0.3 mm vs 4.7 ± 0.3 mm; $P = 0.38$), pulsatility index (3.39 ± 0.25 vs 3 ± 0.25 ; $P = 0.06$) or resistance index (0.75 ± 0.04 vs 0.79 ± 0.04 ; $P = 0.57$).

The pulsatility index was not different between Group A and B on days 8 (3.23 ± 0.27 vs 3.69 ± 0.34 ; $P = 0.29$) or 10 (3.53 ± 0.42 vs 3.86 ± 0.40 ; $P = 0.61$).

Additionally, the size of the leading follicle was not different between Group A and B on days 4 (8.15 ± 0.36 mm vs. 7.8 ± 0.50 mm; $P = 0.94$), 8 (15.38 ± 0.64 mm vs. 14.29 ± 0.81 mm; $P = 0.26$) or 10 (18.65 ± 0.77 mm vs 17.28 ± 0.92 mm; $P = 0.33$). The estradiol levels were between cycles A and B on days 4, 6 and 10 were not significant.

Figure 2 shows that the endometrial thickness was similar between Group A and B on day 4. However, a noticeable difference in the endometrial thickness between cycles A and B on days 8 and 10. In comparison to day 4, endometrial thickness increased on days 8 and 10 of both Group A and Group B (7.5 ± 2.1 mm vs 5.5 ± 1.2 mm, $P < 0.0002$ and 8.9 ± 1.8 mm vs 6.3 ± 1.8 mm, $P < 0.0002$ for days 8 and 10, respectively), and this improvement was more pronounced in the cycle that included tadalafil.

Similarly, the resistance index was not different between cycles A and B on day 4 (Fig. 3), but a tendency of a difference was observed on day 8, and a difference was observed on day 10. A progressive decrease in the resistance index was observed in Cycle A, but not in Cycle B, from day 8 (0.77 ± 0.15 vs 0.85 ± 0.18 , $P = 0.059$) to day 10 (0.74 ± 0.20 vs 0.87 ± 0.14 , $P < 0.017$). In summary, we found a progressive decrease in the resistance index and a remarkable increase in the endometrial thickness when tadalafil was used (Fig. 3).

Discussion

In the present study, we assessed the effects of administering a phosphodiesterase 5 (PDE 5) inhibitor aiming to increase endometrial thickness. Such vasodilators have been used successfully in patients with impaired endometrial growth due to structural damage to the endometrium.

Previously, sildenafil was shown to improve endometrial development (Sher and Fisch, 2000, 2002). However, the effect of using these vasodilator drugs in patients with inadequate endometrial growth, which is not due to intrinsic damage but due to the negative effect of clomiphene citrate treatment on the endometrial growth has not been established yet. Therefore, we examined the effects of the

CONSORT 2010 Flow Diagram

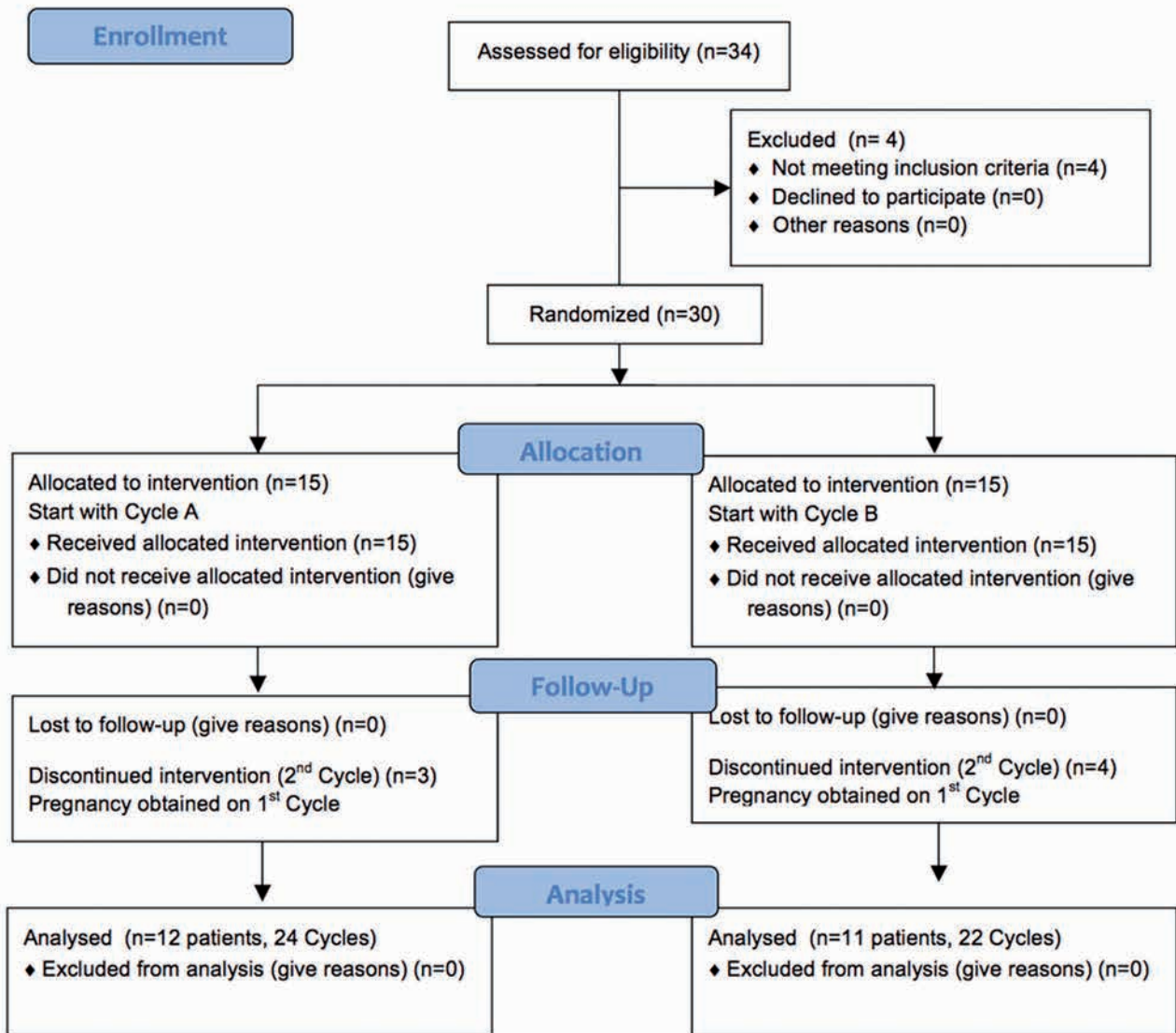


Fig. 1. — Cross-over randomized study: flow-diagram of the study

addition of tadalafil to the classic protocol of ovarian stimulation with clomiphene citrate. Tadalafil was selected because of its longer half-life compared to sildenafil, which may result in a more sustained effect with fewer side effects.

This investigation aimed to limit the confounding variables as much as possible by having each patient be her own control in alternating cycles and by not

altering ovarian stimulation regardless of patient response. Furthermore, the patients were randomized in Group A or Group B with and without the PDE 5 inhibitor, respectively. Additionally the Doppler ultrasound measurement settings were the same for all patients, and the Doppler ultrasound measurements were made in the same manner without changing any of the ultrasound settings.

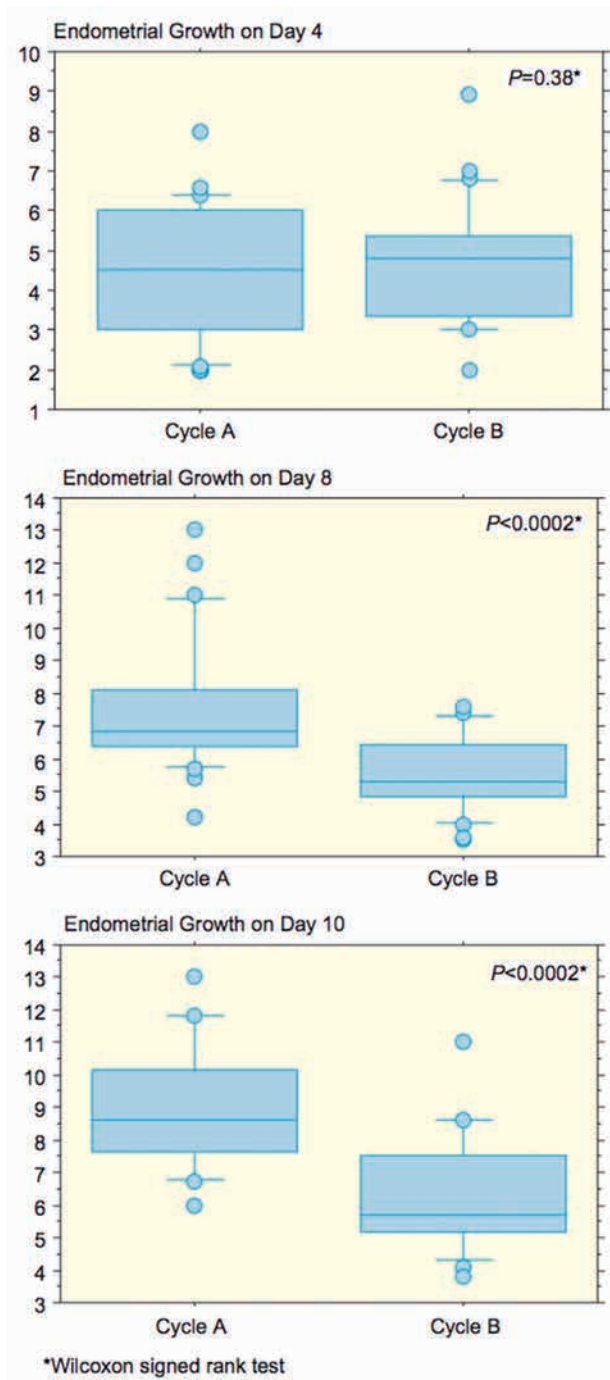


Fig. 2. — Comparison of endometrial growth on Day 4, 8 and 10 between Group A (cycle A) and Group B (cycle B).

Furthermore, the researchers tried to avoid any bias when collecting the Doppler ultrasound measurements. The ultrasound measurements performed by the same blinded operator.

We studied the possible effect of the addition of tadalafil to an ovarian stimulation protocol with clomiphene citrate. We focused on the effect of tadalafil on the endometrium by observing the endometrial thickness as well as the pulsatility index and resistance index of the uterine arteries.

The results we obtained are in line with previous studies that showed that a higher follicular vascular flow provided a significant benefit to ovo-follicular reproductive competence (Lozano et al., 2007).

Out of the 30 patients included, 23 completed both cycles and were included in the analysis of the comparative variables. Additionally, three measurement time points were used: baseline (day 4), at which time similar characteristics should be found for both cycles, day 8, at which time the first noticeable differences between cycles A and B could be observed and day 10 at which time the effect of tadalafil before the end of ovarian stimulation could be observed.

With the addition of a phosphodiesterase PDE5 inhibitor, we found no effect on the pulsatility index at any of the 3 measurement time points. This was in contrast to the findings of other researchers (Sher and Fisch, 2002). It is possible that the acute effect of drugs on the pulsatility index is more noticeable for fast and short-acting drugs such as sildenafil.

The resistance index was also not affected by tadalafil at baseline but a strong trend of a decrease in the resistance index with tadalafil was observed on day 8 of the cycle ($P = 0.059$), and gradually a remarkable decrease was observed on day 10 of the cycle ($P = 0.017$). These changes were less pronounced in other studies that used of short-acting agents.

An important finding of this study was the improvement in endometrial development with the addition of tadalafil to the clomiphene ovarian stimulation protocol. With tadalafil, notable increases in endometrial growth were observed on cycle days 8 ($P < 0.0002$) and 10 ($P < 0.0002$).

However, even though the speed of the follicular growth of the leading follicle was not affected by tadalafil, the production of estradiol tended to respond faster on day 8 for the cycle with tadalafil ($P = 0.06$) in conjunction with a progressive decrease in the resistance index, resulting in proper endometrial development and a rapid response of the healthy endometrium. Of note, follicular growth was adequate in both cycles and the difference in estradiol production between the two cycles disappeared on day 10 of the cycles.

Even when the preliminary design of this study did not include pregnancy outcome, 10 pregnancies were achieved for an overall pregnancy rate of 18.86% per cycle. Six pregnancies were achieved under clomiphene citrate alone (22.2%) and 4 pregnancies were achieved under tadalafil and clomiphene (15.3%).

A better endometrium thickness but a similar pregnancy rates was observed in this study when adding tadalafil to the stimulation protocol.

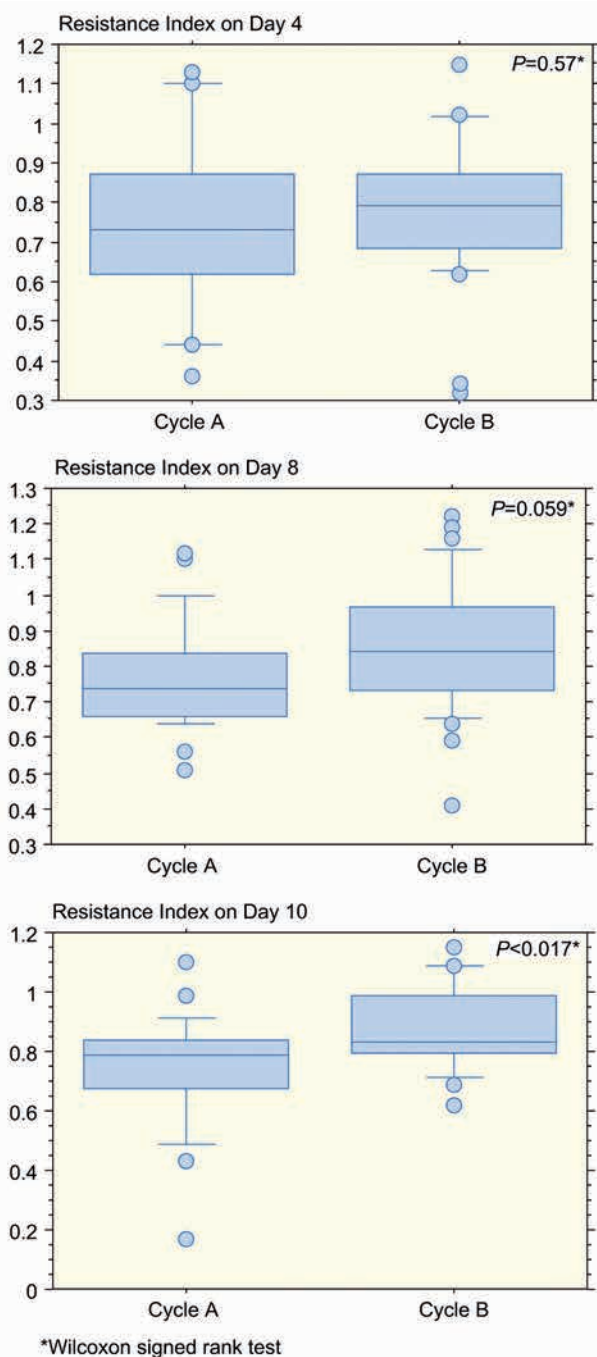


Fig. 3. — Comparison of resistance index (RI) on Day 4, 8 and 10 between Group A (cycle A) and Group B (cycle B).

This is the first study to examine endometrial growth in patients with healthy endometria taking tadalafil in addition to clomiphene citrate for ovarian stimulation. In the present study effects of tadalafil could be observed, especially a decrease in the resistance index. These findings must be verified by other studies and in other population groups.

This study provides an important basis for establishing new hypotheses and performing new studies on the management of vascular dynamics to aid in reproductive competence.

In the present study, we observed better endometrial growth in cycles with tadalafil compared to cycles with clomiphene citrate alone. We also found a progressive decrease in the resistance index in the uterine arteries without changes in follicular development in the cycles with tadalafil. No significant difference in pregnancy rate between the two groups could be observed in this small series of patients.

References

- Abdalla HI, Brooks AA, Johnson MR et al. Endometrial thickness: a predictor of implantation in ovum recipients? *Hum Reprod.*1994;9:363-5.
- Dehghani Firouzabadi R, Davar RR, F. Hojjat F et al. Effect of sildenafil citrate on endometrial preparation and outcome of frozen-thawed embryo transfer cycles: a randomized clinical trial. *Iran J Reprod Med.* 2013;11:151-8.
- Dessolle L, Darai E. Cornet D et al. Determinants of pregnancy rate in the donor oocyte model: a multivariate analysis of 450 frozen-thawed embryo transfers. *Hum Reprod.* 2009;24:3082-9.
- El Refaey A, Selem A, Badawy A. Combined coenzyme Q10 and clomiphene citrate for ovulation induction in clomiphene-citrate-resistant polycystic ovary syndrome. *Reprod Biomed Online.* 2014;29:119-24.
- Fanchin R, Schonauer LM, Righini C et al. Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod.* 2003;18:323-7.
- Fanchin R, Mendez Lozano DH, Schonauer LM et al. Hormonal manipulations in the luteal phase to coordinate subsequent antral follicle growth during ovarian stimulation. *Reprod Biomed Online.* 2005;10:721-8.
- Fanchin R, Louafi N, Mendez Lozano DH et al. Per-follicle measurements indicate that anti-mullerian hormone secretion is modulated by the extent of follicular development and luteinization and may reflect qualitatively the ovarian follicular status. *Fertil Steril.* 2005;84:167-73.
- Fanchin R, Mendez Lozano DH, Frydman N et al. Anti-Mullerian hormone concentrations in the follicular fluid of the preovulatory follicle are predictive of the implantation potential of the ensuing embryo obtained by in vitro fertilization. *J Clin Endocrinol Metab.* 2007;92:1796-802.
- Forgue ST, Patterson BE, Bedding AW et al. Tadalafil pharmacokinetics in healthy subjects. *Br J Clin Pharmacol.* 2006;61:280-8.
- Gleicher N, Kim A, Michaeli T et al. A pilot cohort study of granulocyte colony-stimulating factor in the treatment of unresponsive thin endometrium resistant to standard therapies. *Hum Reprod.* 2013;28:172-7.
- Jirge PR, Patil RS. Comparison of endocrine and ultrasound profiles during ovulation induction with clomiphene citrate and letrozole in ovulatory volunteer women. *Fertil Steril.* 2010;93:174-83.
- Kafy S, Tulandi T. New advances in ovulation induction. *Curr Opin Obstet Gynecol.* 2007;19:248-52.
- Lebovitz O, Orvieto R. Treating patients with “thin” endometrium – an ongoing challenge. *Gynecol Endocrinol.* 2014;30:409-14.
- Lozano DH, Frydman N, Levaillant JM et al. The 3D vascular status of the follicle after HCG administration is qualitatively rather than quantitatively associated with its reproductive competence. *Hum Reprod.* 2007a;22:1095-9.
- Mendez Lozano DH, Brum Scheffer J, Frydman N et al. Optimal reproductive competence of oocytes retrieved through follicular flushing in minimal stimulation IVF. *Reprod Biomed Online.* 2008;16:119-23.

- Palatnik A, Strawn E, Szabo A et al. What is the optimal follicular size before triggering ovulation in intrauterine insemination cycles with clomiphene citrate or letrozole? An analysis of 988 cycles. *Fertil Steril.* 2012;97:1089-94 e1-3.
- Paulus WE, Strehler E, Zhang M et al. Benefit of vaginal sildenafil citrate in assisted reproduction therapy. *Fertil Steril.* 2002;77:846-7.
- Reynolds K, Khoury J, Sosnowski J et al. Comparison of the effect of tamoxifen on endometrial thickness in women with thin endometrium (< 7mm) undergoing ovulation induction with clomiphene citrate. *Fertil Steril.* 2010;93:2091-3.
- Senturk LM, Erel CT. Thin endometrium in assisted reproductive technology. *Curr Opin Obstet Gynecol.* 2008; 20:221-8.
- Sher G, Fisch JD. Vaginal sildenafil (Viagra): a preliminary report of a novel method to improve uterine artery blood flow and endometrial development in patients undergoing IVF. *Hum Reprod.* 2000;15:806-9.
- Sher G, Fisch JD. Effect of vaginal sildenafil on the outcome of in vitro fertilization (IVF) after multiple IVF failures attributed to poor endometrial development. *Fertil Steril.* 2002;78:1073-6.
- Takasaki A, Tamura H, Miwa I et al. Endometrial growth and uterine blood flow: a pilot study for improving endometrial thickness in the patients with a thin endometrium. *Fertil Steril.* 2010;93: 1851-8.
- Wrishko R, Sorsaburu S, Wong D et al. Safety, efficacy, and pharmacokinetic overview of low-dose daily administration of tadalafil. *J Sex Med.* 2009;6:2039-48.
- Zinger M, Liu JH, Thomas MA. Successful use of vaginal sildenafil citrate in two infertility patients with Asherman's syndrome. *J Womens Health (Larchmt).* 2006;15:442-4.