Original Articles

Neither sildenafil nor vaginal estradiol improves endometrial thickness in women with thin endometria after taking oral estradiol in graduating dosages

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Summary

Purpose: To determine if sildenafil improves endometrial thickness better than vaginal estradiol (E2) in women with a history of thin endometria.

Methods: Women failing to attain an 8 mm endometrial thickness on either the oocyte retrieval cycle or their first frozen embryo transfer (ET) despite an oral graduated E2 regimen were treated again with graduated oral E2 and were also randomly assigned to vaginal sildenafil or vaginal E2 therapy. Endometrial thickness was compared between the groups.

Results: Neither vaginal E2 nor sildenafil significantly improved endometrial thickness or blood flow in the subsequent frozen ET-cycle.

Conclusions: These data fail to corroborate previous claims that 25 mg sildenafil four times daily intravaginally can improve endometrial thickness.

Key words: Endometrial blood flow; Endometrial thickness; Estradiol; Frozen embryo transfer; Sildenafil.

Introduction

There have been data suggesting lower pregnancy rates are associated with thin pre-ovulatory endometria in in vitro fertilization-embryo transfer (IVF-ET) cycles [1-3]. There has also been data showing reduced fecundity in oocyte recipients with thin endometria where only a regimen of graduating dosages of oral estradiol (E2) was given [4]. With modern IVF techniques, the minimal endometrial requirements may be power today than when these studies were conducted. Still, most IVF centers would agree there remains a subset of women who consistently have a thin endometrium and a low pregnancy rate despite the use of therapies to improve endometrial thickness [5].

Recently, a novel method to improve endometrial thickness and blood flow has been reported using vaginal sildenafil [6, 7]. Other studies have demonstrated that improvement in these same parameters may be achieved by administering E2 vaginally [8-10]. The prospective randomized study presented here was designed to further evaluate whether sildenafil increases endometrial thickness more than vaginal E2 in women previously failing to attain an adequate endometrial thickness for successful frozen ET despite undergoing a graduated oral E2 protocol.

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Materials and Methods

The design of the study was a two-arm parallel design. One arm included those women who received graduated oral E2 with sildenafil. The other arm included those patients who received graduated oral E2 with vaginal E2. The original design did not call for the patients to cross-over to the other treatment arm, but some women decided to proceed to the second treatment arm if they failed the first.

Patient eligibility for this study included those women who failed to attain an endometrial thickness of 8 mm or greater on their first controlled ovarian hyperstimulation (COH) cycle and on one or more prior attempts at frozen ET. The patients were informed that a recent report suggested improved endometrial thickness could be attained by the use of sildenafil [6]. The patients were also informed of the data suggesting vaginal E2 could improve endometrial thickness [8-10]. Based our experience they were advised that repeating the same oral E2 regimen used previously was not likely to result in a better endometrial thickness. It was also explained that if they participated in the study and did not receive sildenafil along with the previous oral E2 regimen, they would receive 2 mg vaginal E2 plus the previous oral E2 regimen of 2 mg E2 x 5 days, 4 mg x 4 days and 6 mg x 5 days.

Patients willing to be part of this study approved by the Institutional Review Board of Robert Wood Johnson Medical School at Camden of signed informed consent forms. The women were then randomly assigned (using a random numbers table) to the group that received the same oral estrogen regimen plus intravaginal sildenafil or the oral estrogen regimen plus vaginal E2. The sildenafil citrate (Viagra) in the form of vaginal suppositories was prescribed at 25 mg four times daily from days three to nine of the menstrual cycle with a vaginal wash on day 10 as previously described [6]. The group receiving vaginal E2 was given 2 mg twice daily from day 2 until maximum endometrial thickness was attained.

If the maximum thickness acceptable for ET was achieved by day 20, ET occurred four days later; otherwise the cycle was cancelled at that time. All patients who went on to ET had progesterone (P) support in the luteal phase (200 mg twice daily P vaginal suppositories and 100 mg P in oil daily).

Cycle monitoring included serum E2 and P levels, sonographic evaluation of endometrial thickness, echo pattern, and color Doppler studies to determine uterine artery impedance and uterine perfusion. Mean endometrial thickness was reported for all previous failed cycles prior to the cycle that initiated the randomized therapy for this study. Patients who had ET cancelled in one treatment arm were offered the alternative treatment regimen for their next cycle. Cycle monitoring was identical for this treatment arm.

Endometrial thickness was measured by placing electronic calipers on the outer walls of the endometrium at the widest diameter in the longitudinal axis.

Color Doppler analysis of uterine artery impedance, as expressed in measures of pulsatility index (PI) and resistance index (RI) was performed starting on day 14 of E2 therapy on all patients seen in our primary facility. Color Doppler signals were obtained from the right and left ascending branches of the uterine arteries at the level of the internal os. Once visualized, a pulsed Doppler range gate was placed over each artery to obtain velocity waveforms. When multiple consecutive images were obtained, the PI and RI were measured by electronically tracing the waveform. The average of the right and left measurements was included in the analysis.

Color power-Doppler sonography to detect uterine and endometrial vascularization was also performed on all patients seen in our primary facility. Vascularization was classified according to the visualization of the depth of power-Doppler signals seen within the uterus; grade 1 was classified as flow seen only in the outer myometrium, grade 2 flow was seen in the myometrium extending to, but not including the walls of the endometrium, grade 3 flow extended into the walls of the endometrium, and grade 4 flow was seen within the endometrial cavity.

All sonograms were performed on a GE Logic 400 (General Electric Medical Systems, Milwaukee, WI). All measurements of endometrial thickness, PI, RI, and power Doppler grading, were performed by one sonographer.

Serum hormonal levels of E2 were obtained each time a sonographic evaluation was performed.

Due to the small sample size non-parametric hypothesis testing was used in the comparisons (Mann-Whitney U and Wilcoxon test). A p value of .05 was used to determine significance.

Results

A total of 20 women were initially entered in the study but four dropped out after randomization and signing informed consent forms and are not included in this analysis.

The endometrial thickness on the cycle prior to entering the study ranged from 5-7.9 mm with a median of 7 mm. The PI ranged from 1.15 to 3.38 with a median of 2.78; the RI ranged from .67 to .92 with a median of .90. The age of the patients ranged from 34 to 45 with a median age of 39. All of the women using their own oocytes were under the age of 41 at the time of oocyte retrieval and three of the women used donor oocytes. All the women in this study had no known etiology for their previous thin endometrium. Causes of infertility included male factor (n = 5), elevated follicle stimulating hormone (FSH) (n = 4), tubal (n = 4), and idiopathic (n = 3).

Nine women were randomized to the vaginal sildenafil (VS) protocol in their first cycle and seven to vaginal E2 (VE). Of the VS group, three women completed both study arms: six women completed a cross-over cycle in the VE group (Table 1).

There were no significant differences in the median endometrial thickness, PI or RI in the cycle immediately prior to entering the study and the first study cycle within each treatment group (Table 2) (Wilcoxin signed rank test). Serum estradiol levels, measured on the day prior to P administration, were not significantly different in the prior cycle, VS or VE cycle (Table 2).

There were also no significant differences in the sonographic parameters between the treatment groups (Mann-Whitney U test).

The distribution of subendometrial blood flow (SEF) patterns in the prior study cycles were the same within each group. The distribution of SEF patterns in the study cycle was the same for both treatment groups.

Table 1. — *Descriptive statistics for endometrial parameters on treatment cycles.*

Group	Treatment ET	Cross-over ET	Treatment PI	Treatment RI
Vaginal Estrogen				
Mean endometrial thickness	7.14	6.83	2.31	0.84
Standard error of mean	0.26	0.31	0.42	0.006
Median endometrial thickness	7.00	7.00	2.57	0.88
Standard deviation	0.69	0.752	0.85	0.114
Minimum	6	6	1.15	0.67
Maximum	8	8	2.97	0.92
Vaginal Sildenafil				
Mean endometrial thickness	6.44	7.33	2.63	0.885
Standard error of mean	0.38	0.67	0.17	0.001
Median endometrial thickness	6.00	8.00	2.78	0.905
Standard deviation	1.13	1.15	0.522	0.003
Minimum	5	6	1.78	0.82
Maximum	8	8	3.38	0.92

Table 2. — *Comparison of endometrial parameters by therapy.*

	Prior Frozen ET Cycle[a,b]	First Study Cycle[a,b]
Sildenafil Group		
Endometrial thickness (mm)[c]	6.0 (4.0-7.0)	6.0 (5.0-8.0)
Pulsatility index[c]	2.78 (1.78-3.38)	2.40 (1.74-3.40)
Resistance index[c]	0.90 (0.82-0.92)	0.87 (0.80-0.95)
Blood flow grade	Distribution	Distribution
1	2	1
2	1	3
3	2	2
4	0	1
E2 (pg/ml)	1480 (1141-2657)	1310 (219-3756)
Vaginal E2 Group		
Endometrial thickness (mm)[c]	7.0 (6.0-7.0)	7.0 (6.0-8.0)
Pulsatility index[c]	2.57 (1.12-2.97)	2.33 (1.23-3.47)
Resistance index[c]	0.83 (0.67-0.92)	0.87 (0.83-0.94)
Blood flow grade	Distribution	Distribution
1	1	0
2	2	1
3	1	2
4	0	2
E2 (pg/ml)	1125 (215-3688)	2144 (1146-4034)

[a] $p > 0.05$ comparing group 1 to group 2, Mann-Whitney U test.
[b] $p > 0.05$ comparing previous cycle to current cycle, Wilcoxin-signed rank test.
[c] Data presented as median (minimum, maximum).

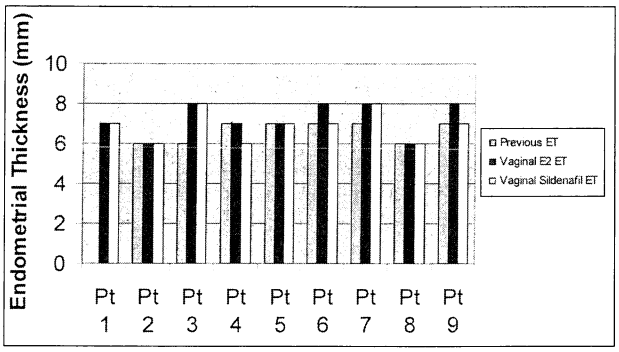

Figure 1. — Cross-over data.

Figure 1 presents the actual data for the nine women who crossed over. No significance was demonstrated when comparing the sildenafil response to the vaginal E2 response or when comparing either treatment arm to the previous failed cycle.

Discussion

These data suggest that neither sildenafil therapy nor vaginal E2 improves maximum endometrial thickness or blood flow parameters to any significant extent in women with a history of thin endometrial lining on prior ET attempts. Contrary to our results, Sher and Fisch reported in a retrospective cohort analysis that sildenafil significantly improved endometrial lining in women with prior IVF failures with thin endometria [7]. Unlike the present study, their study was a retrospective cohort analysis of women with at least two prior IVF failures. Given the study=s design [7], there is no way of knowing for sure if these women would have achieved adequate endometrium (≥ 9 mm as defined by the authors) if no intervention was done at all on the subsequent IVF cycle. This study differs in that we prospectively looked at sildenafil vs vaginal E2 to try to better isolate the effect of these treatment protocols on the endometrium. It should also be noted that this study used oral E2 in contrast to the estradiol valerate given IM used in the studies by Sher & Fisch [6, 7]. Our study also differs from the studies evaluating just vaginal E2, since we used oral E2 in combination with the vaginal route and our patient population was restricted to those with a history of poor endometrial response [8-10].

Though our results fail to show a positive impact on endometrial thickness with sildenafil treatment, many questions on nitric oxide modulation of the uterine lining still need to be addressed. There are data suggesting that follicle maturing drugs may create a hostile uterine environment, thus adversely affecting implantation and pregnancy rates [11-14]. The study by Sher and Fisch [7] evaluated women undergoing COH cycles along with their sildenafil treatment. The design of the present study was different, i.e., we proceeded with oocyte retrieval and despite thin endometria cryopreserved all embryos and then attempted to improve the lining for subsequent frozen ET through exogenous estrogen in high doses plus the use of vaginal estradiol or sildenafil. It is possible that new studies may show that the thickness of the endometrium is not as important for frozen ET where COH is not administered. If that is found to be the case, then it would not matter very much that neither sildenafil nor vaginal E2 are likely to improve endometrial thickness for frozen ETs.

The possibility remains, however, that sildenafil treatment may produce improvements in those endometria exposed to hostile affects of COH cycles thus resulting in improved IVF outcome. To test this hypothesis, it would require a prospective study where ET would be performed following sildenafil treatment vs control therapy regardless of the thickness of the endometrium. Neither the present study nor the retrospective study by Sher and Fisch was designed to evaluate this possible effect.

Sher and Fisch also suggest that sildenafil treatment may fail if the basal layer of the endometrium is irreversibly damaged due to a history of pregnancy-related endometritis [7]. The present study did not include any patients with this history, as the thin endometrium was unexplained in almost all cases.

The women in the present study included those over 40 and the average age was 40.8. Sher and Fisch's study only included women under 40 with an average age of 35.8 [7]. Hence, the possibility remains that age is a confounding variable. Perhaps, women with thin endometria who are younger are more responsive to therapies such as sildenafil and vaginal estradiol.

More importantly, before more time and money are spent on prospective studies to evaluate the benefit of therapies like sildenafil and vaginal estradiol to improve the thickness of thin endometria, it is important to determine what is the critical thickness below which pregnancy rates suffer in the modern era of IVF. This needs to be done independently for COH-oocyte retrieval cycles and frozen ET or donor-oocyte transfers. Most of the data addressing the adverse affect of the thin endometria was published in the era where the embryos were not as "hearty" as the ones today (due to the improvements in the IVF laboratory) [1-5, 15-28].

Probably before more studies are conducted to see if certain therapies can improve endometrial thickness, repeat evaluation in large populations should be made to determine what is the critical endometrial thickness level below which reduced pregnancy rates are seen. This should be done independently for IVF-ET cycles following COH, frozen ET or donor oocyte cycles following graduated estrogen/P replacement, and for the latter, in cycles using clomiphene citrate or natural cycles.

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