

Vaginal sildenafil (Viagra): a preliminary report of a novel method to improve uterine artery blood flow and endometrial development in patients undergoing IVF

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Endometrial growth is thought to depend on uterine artery blood flow and the importance of endometrial development on in-vitro fertilization (IVF) outcome has been previously reported. Nitric oxide (NO) relaxes vascular smooth muscle through a cGMP-mediated pathway and NO synthase isoforms have been identified in the uterus. Sildenafil citrate (Viagra), a type 5-specific phosphodiesterase inhibitor, augments the vasodilatory effects of NO by preventing the degradation of cGMP. In this preliminary report we describe the use of vaginal sildenafil to improve uterine artery blood flow and sonographic endometrial appearance in four patients with prior failed assisted reproductive cycles due to poor endometrial response. The uterine artery pulsatility index (PI) was measured in a mock cycle after pituitary down-regulation with Lupron. The PI was decreased after 7 days of sildenafil (indicating increased blood flow) and returned to baseline following treatment with placebo. The combination of sildenafil and oestradiol valerate improved blood flow and endometrial thickness in all patients. These findings were reproduced in an ensuing gonadotrophin-stimulated cycle. Three of the four patients conceived. Although greater numbers of patients and randomized evaluation are needed to validate this treatment, vaginal sildenafil may be effective for improving uterine artery blood flow and endometrial development in IVF patients with prior poor endometrial response.

Key words: endometrium/in-vitro fertilization/sildenafil/uterine artery blood flow

Introduction

A trilaminar sonographic endometrial pattern of ≥ 8 mm on the day of human chorionic gonadotrophin (HCG) has been shown to be correlated with a high chance of pregnancy in patients being treated with IVF (Gonen *et al.*, 1990; Sher *et al.*, 1991). Several regimens have been proposed to improve a poor endometrial response, including treatment with oestrogens and low dose aspirin (Sher *et al.*, 1993; Weckstein *et al.*, 1997). In the past few years, much interest has been focused on the role of nitric oxide (NO) as a modulator of uterine blood flow (Amit *et al.*, 1998; Cameron and Campbell, 1998).

Previous animal research has demonstrated that NO release leads to the relaxation of vascular smooth muscle through a cGMP-mediated pathway (Ballard *et al.*, 1998). Endothelial and inducible NO synthase isoforms have also been identified in the vascular endothelium of human endometrium and myometrium (Telfer *et al.*, 1997). Based on a recent report (Smith and Brien, 1998), and on anecdotal obstetric uses of nitroglycerine (NTG), a NO donor, for uterine relaxation and tocolysis, we speculated that NTG could be used to improve endometrial development. Treatment with NTG patches did produce a trilaminar sonographic pattern and increased endometrial thickness, but was impractical due to an unacceptably high rate of headaches, nausea and hypotension.

Phosphodiesterase (PDE) is a family of isoenzymes that hydrolyse cAMP and cGMP. Specific inhibitors of PDE subtypes have been identified that can augment the effects of cyclic nucleotides on target tissues, such as human spermatozoa (Fisch *et al.*, 1998). Sildenafil citrate (Viagra) is a newly developed, type 5-specific PDE inhibitor that prevents the breakdown of cGMP and potentiates the effects of NO on vascular smooth muscle. Since its introduction in 1997, sildenafil has been used with great success in the treatment of male erectile dysfunction (Boolell *et al.*, 1996), but as yet no one has evaluated its effects in women.

The availability of sildenafil has enabled us to reap the benefits of NO on the uterus, while minimizing side-effects. In this report we describe four patients treated with IVF, who each had at least three previous failed assisted reproduction cycles (IVF and/or intrauterine insemination), with good ovarian stimulation and a poor endometrial response, who showed marked improvement in uterine artery blood flow and endometrial development following treatment with sildenafil.

Materials and methods

Four patients were enrolled in this observational study. A cross-over design using each patient as her own control was employed. Prior to gonadotrophin stimulation, all patients were evaluated in a mock cycle after pituitary down-regulation for 10–14 days with Lupron (TAP Pharmaceuticals, Inc., Deerfield, IL, USA). Baseline measurement of uterine artery blood flow (pulsatility index, PI) was recorded using Doppler ultrasound (LOGIQ 400, GE Medical Systems, Milwaukee, WI, USA) (Figure 1A).

Patients self-administered sildenafil (Pfizer Inc., New York, NY, USA) suppositories, prepared for us from oral tablets by a local pharmacy, 25 mg intravaginally, four times per day for 7 days. PI and endometrial thickness were evaluated at 168 h. Sildenafil was then discontinued and each patient received placebo suppositories for 7 days and blood flow parameters were remeasured. Each patient then received a combination of sildenafil suppositories 25 mg intravaginally

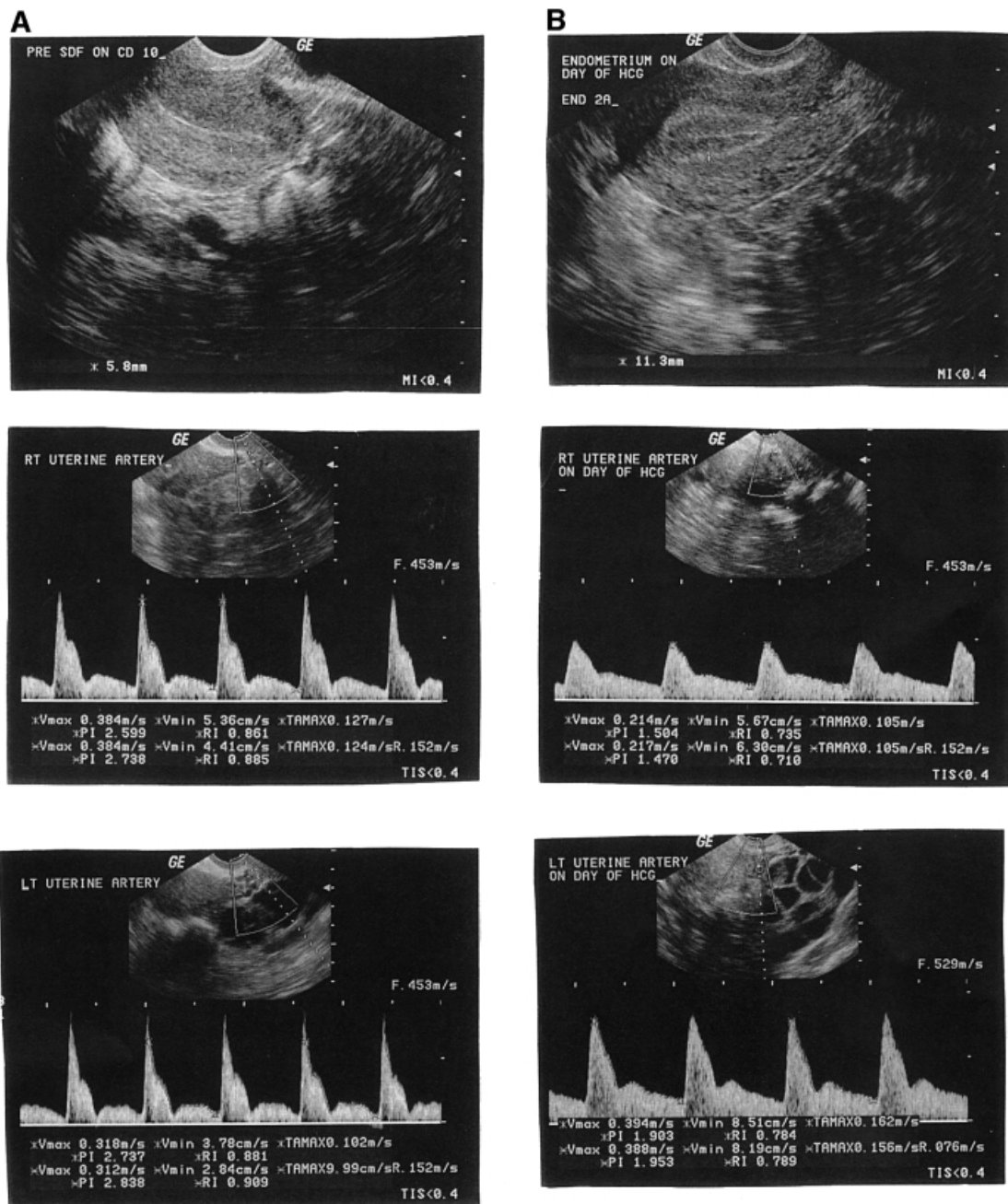


Figure 1. (A) Baseline sonographic evaluation of endometrial appearance and Doppler flow of the uterine arteries in patient 1 after pituitary down-regulation with Lupron for 10 days. The endometrium is thin and the pulsatility index (PI) is high. (B) Sonographic endometrial appearance and Doppler flow of the uterine arteries in patient 1 on the day of human chorionic gonadotrophin injection after treatment with sildenafil and FSH for 10 days. The endometrium was thick (11 mm) and displays a trilaminar pattern. The PI was decreased compared to the baseline, indicating increased uterine blood flow.

four times per day and oestradiol valerate 4 mg i.m., twice per week for 8 days, at which time PI and sonographic endometrial appearance were re-evaluated. Sildenafil and oestradiol were then stopped, while the Lupron was continued and the patient experienced a menstrual bleed.

Controlled ovarian hyperstimulation was then begun using Gonal-F (Serono Laboratories, Norwell, MA, USA) in an initial dose of 300–375 IU, which was decreased by 150 IU on the third day of stimulation. At the same time sildenafil suppositories, 25 mg four times per day, were restarted and were continued until the day of HCG administration (8–12 days). Oestradiol concentrations and ultrasonographic monitoring of folliculogenesis, endometrial pattern

and uterine artery blood flow were begun on cycle day 9 (Figure 1B). Ovulation was triggered with 10 000 IU HCG (Profasi; Serono) when two lead follicles were ≥ 18 mm in diameter and half the remainder were at least 15 mm. Oocytes were retrieved transvaginally under ultrasound guidance, 35 h later.

Metaphase II oocytes underwent intracytoplasmic sperm injection (ICSI) 4–5 h after retrieval. Resulting embryos were cultured to the blastocyst stage, at which point two embryos were transferred to the uterus. Progesterone in oil, 50 mg i.m. twice daily, was used for luteal support. Serum β HCG concentrations were obtained 11 and 13 days after oocyte retrieval. A viable pregnancy was defined by fetal cardiac activity on ultrasound examination at 6–7 weeks of gestation.

Table I. Characteristics of patients treated with sildenafil

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	34	37	31	34
Diagnosis	DES uterus	Adenomyosis	Unexplained	Synechiae/septum resected
Prior treatment	Failed IVF (<i>n</i> = 3)	Failed IUI (<i>n</i> = 4) Failed IVF (<i>n</i> = 3)	Failed IUI (<i>n</i> = 6) Failed IVF (<i>n</i> = 1)	Failed IUI (<i>n</i> = 3)
Best prior endometrial thickness (mm) ^a	6	6	7	5
Outcome	Pregnant	Pregnant	Pregnant	Not pregnant

^aAt each examination, multiple measurements of the lining were made by the same person until a consensus was reached. The error was >0.1 mm but <0.5 mm. Therefore measurements were rounded to the nearest whole number.
DES = diethylstilboestrol; IUI = intrauterine insemination.

Table II. The effect of sildenafil on uterine artery blood flow and endometrial thickness

Treatment	Side	Patient 1		Patient 2		Patient 3		Patient 4	
		PI (mm)	ET	PI (mm)	ET	PI (mm)	ET	PI (mm)	ET
L×10–14 days (baseline)	R	2.7	4	3.2	3	3.4	3	2.6	3
	L	2.8		3.4		3.3		2.0	
L/sildenafil ×7 days	R	1.6	3	2.2	3	2.7	3	1.9	3
	L	1.8		2.2		2.6		1.6	
L/placebo ×7 days	R	2.9	3	3.0	3	3.3	2	2.7	3
	L	3.0		2.9		3.5		2.6	
L/sildenafil/E ₂ V ×8 days	R	1.6	10	2.0	10	2.9	11	1.1	7
	L	1.8		2.1		2.6		1.7	
L/sildenafil/FSH ×8–12 days (until day of HCG)	R	1.5	11	1.9	10	2.3	12	1.1	8
	L	1.9		2.1		2.5		1.5	

PI = pulsatility index; ET = endometrial thickness; L = Lupron; E₂V = oestradiol valerate; FSH = follicle stimulating hormone (Gonal-F); HCG = human chorionic gonadotrophin; R = right; L = left.

Results

The four patients ranged in age between 31 and 37 years (Table I). They suffered from a range of uterine disorders including, diethylstilboestrol (DES) exposure, adenomyosis, and intrauterine synechiae following a hysteroscopic septoplasty. Prior to treatment with sildenafil all four patients experienced multiple failed cycles of IVF and/or intrauterine insemination (IUI) with a maximum endometrial thickness of <8 mm.

The PI ranged between 2.0 and 3.4 after down-regulation with Lupron and decreased to between 1.5 and 2.7 after 7 days of sildenafil, reflecting increased diastolic flow (Figure 1 and Table II). The PI returned to baseline after 7 days of placebo. The PI was again markedly decreased following 7 days of sildenafil/oestradiol valerate and the endometrial thickness developed to >10 mm in three of the four patients. These findings were reproduced in an ensuing gonadotrophin-stimulated cycle (Table II). Three patients ultimately became pregnant. One patient could not be stimulated to produce an endometrial lining of >8 mm, despite improvement of her PI. She had a history of synechiae following the resection of a uterine septum and this patient was the only one who did not conceive. There were no complaints of side-effects from any of the four patients.

Discussion

The importance of endometrial appearance as a predictor of outcome in patients treated with IVF is well established (Gonen and Casper, 1990; Sher *et al.*, 1991, 1993). However, treatment with oestrogens alone does not appear to improve pregnancy rates significantly in patients with poor endometrial response. NO is recognized as a mediator of vascular smooth muscle dilatation in many areas of the body. NTG has long been used for its vasodilatory properties in the treatment of angina, as well as obstetrically, to achieve tocolysis and uterine relaxation. We have used NTG successfully to improve uterine artery blood flow and endometrial lining in IVF patients with a previous poor response. However, we experienced a high rate of side-effects, including hypotension and headaches. The use of intravaginal sildenafil suppositories made it possible to decrease the incidence of these side-effects by delivering medication in close proximity to the target organ.

All of the patients reported here had at least three prior failed assisted reproduction cycles with good ovarian stimulation, and none achieved a lining >8 mm despite previous treatment with oestrogen. Using a cross-over study design, we demonstrated the ability of sildenafil to modulate uterine artery blood flow and improve endometrial pattern and thickness. Three of the four patients conceived. The one who did not, had

intrauterine synechiae. While her PI did improve with sildenafil, she may have had subtle basal endometrial damage rendering her unresponsive to increases in uterine blood flow. Her case underscores the complexity of endometrial development and demonstrates that no one factor can explain all implantation failures.

While improving uterine blood flow in the proliferative phase, NO may have detrimental effects at the level of the endometrium during the implantation window. The NO-mediated release of cytokines such as tumour necrosis factor- α from activated natural killer cells has been implicated as a cause of implantation failure (Barroso *et al.*, 1998). It may be beneficial to minimize endometrial NO exposure at the time of embryo transfer and we recommend discontinuing sildenafil supplementation on or prior to the day of HCG administration.

The four patients presented here were strictly monitored with a rigorous protocol that used each patient as her own control. Due to the nature of our practice, the ultrasonographer could not be blinded to the treatment conditions. However, the data were collected in the same manner at all evaluations and the manner of data collection did not affect the outcomes. While these numbers are too small to make generalizations regarding pregnancy rates, a definite improvement in uterine artery blood flow and endometrial development was seen in all patients, and three out of four patients conceived after treatment with sildenafil. Since our initial investigation, we have used sildenafil to improve the uterine artery blood flow and endometrial appearance in more than 20 additional patients with promising results. However, before recommending its widespread use, the benefits of sildenafil should be evaluated in a randomized manner and further investigation is warranted.

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