

# The relationship of endometriosis to endometrial sonographic studies prior to administration of human chorionic gonadotrophin in patients undergoing in-vitro fertilization and embryo transfer

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**The objective of this prospective comparative study was to investigate the relationship of endometriosis to endometrial thickness and sonographic echo pattern prior to the administration of human chorionic gonadotrophin (HCG). Patients were matched by age and ovarian stimulation protocol. A total of 210 patients undergoing in-vitro fertilization (IVF) and embryo transfer at a university-related IVF centre were enlisted. Of these, 105 women with laparoscopic confirmation of endometriosis were compared to an equal number of patients with laparoscopic confirmation of no endometriosis. Mean endometrial thickness did not differ between the groups ( $12.7 \pm 2.9$  versus  $12.2 \pm 2.5$  mm). The distribution of echo patterns was also the same, irrespective of diagnosis. Evaluation of clinical pregnancy rates showed no reduction in patients with endometriosis, regardless of stage, nor when comparing patients to controls. Endometriosis has no effect on the endometrial thickness or echo pattern measured by sonography prior to administration of HCG or the pregnancy rates following IVF and embryo transfer. Key words: endometrial thickness/endometriosis/IVF/sonographic echo pattern/sonography**

## Introduction

With the increased use of in-vitro fertilization (IVF) and embryo transfer as a treatment for infertile women with endometriosis, some studies have reported reduced pregnancy rates in patients with moderate to severe endometriosis following IVF and embryo transfer (Matson and Yovich, 1986; Wardle *et al.*, 1986; Oehninger *et al.*, 1988; Yovich *et al.*, 1988). Possible explanations for this reduction have been poorer oocyte quality (Oehninger *et al.*, 1988), difficulties in oocyte retrieval using laparoscopic methods (Oehninger *et al.*, 1988; Yovich *et al.*, 1988), an implantation inhibitory factor in women with severe endometriosis (Yovich *et al.*, 1988), or a reduction in number of oocytes retrieved (Wardle *et al.*, 1986).

The purpose of this study was to investigate the effect of endometriosis on the thickness and echo pattern of the endometrium as measured by transvaginal sonography on the day of human chorionic gonadotrophin (HCG) administration for IVF and embryo transfer. Since many reports (Smith *et al.*, 1984; Gonen *et al.*, 1989, 1990; Check *et al.*, 1991, 1993b,c; Khalifa *et al.*, 1992) have shown a reduced pregnancy rate following IVF and embryo transfer in cases with a thinner endometrium or in cases where the echo pattern was of the homogeneous hyperechogenic pattern on the day of HCG injection, it was postulated that endometriosis has a negative effect on the endometrium, inhibiting endometrial proliferation and implantation.

To study this hypothesis, the sonographic endometrial characteristics of women with endometriosis were compared to those of a control group comprised of women with no endometriosis.

## Materials and methods

A total of 105 IVF cycles performed between November 1991 and July 1993 at the Cooper Center for IVF and embryo transfer, New Jersey, USA, in which the primary cause of infertility was endometriosis, were evaluated. All of these patients had a laparoscopy performed within 1 year of the IVF cycle to confirm diagnosis of endometriosis. Stage determinations were made using the American Fertility Society (AFS, 1985) classification.

Each patient with endometriosis was matched with a patient who had a diagnosis of no endometriosis based on laparoscopic screening; the matched pair both followed the same ovarian stimulation regimen and were within 2 years of age. Patients with endometriosis as the sole infertility factor were matched to patients with either tubal factor or unexplained infertility. Endometriosis patients with multiple infertility factors were matched with patients without endometriosis who had similar additional factors.

Patients were stimulated using either the luteal phase leuprolide acetate/human menopausal gonadotrophin (HMG) protocol (Meldrum *et al.*, 1989), the flare protocol (Garcia *et al.*, 1990) or the clomiphene citrate/HMG protocol in which 100 mg/day of clomiphene citrate was given on days 3-7 together with 75 IU of HMG on days 3-6. The HMG was increased to 150 or 225 IU/day until the day HCG was administered. The decision as to which protocol to use was based on past history of response to stimulation, age of patient and baseline follicular phase follicle stimulating hormone (FSH) concentrations. The dosage of HMG, FSH and/or

clomiphene citrate administered was noted to guarantee the effectiveness of the matching.

Prior to the administration of HCG, careful endometrial sonographic measurements were made using an ATL Ultramark 4 Unit (Advanced Technology Laboratories, Bothell, WA, USA) equipped with a 5 MHz vaginal transducer. Endometrial thickness was measured in millimeters by placing electronic calipers on the outer walls of the endometrium in the longitudinal axis of the uterine body. The endometrial echo patterns visualized sonographically were graded A, B or C using the following criteria: pattern A presented as a triple-line pattern or a multi-layered endometrium in which hyperechogenic outer lines and a well-defined central echogenic line were visualized with hypo-echogenic or black areas seen between these lines; pattern B was an intermediate pattern in which the endometrium had the same echogenicity as the myometrium with a poorly defined central echogenic line; pattern C was an entirely homogeneous, echo-dense endometrium in comparison with the myometrium in which no central echogenic line could be visualized.

On the day of the sonographic studies, serum hormone concentrations were measured for oestradiol and progesterone. The number of follicles  $\geq 15$  mm and the number of follicles  $\geq 18$  mm were also noted. Other variables measured included number of oocytes retrieved, fertilization rate, number of embryos transferred and outcome. A clinical pregnancy was defined as a pregnancy in which an intra-uterine gestational sac was visualized sonographically.

The endometrial thickness, serum hormone concentrations, number of follicles, number of oocytes retrieved, and number of embryos transferred were compared between the study (endometriosis) and control groups using paired *t*-tests. Variables were compared by stage of endometriosis using analysis of variance. The distribution of echo patterns was compared between the groups using  $\chi^2$  analysis. The pregnancy rates achieved by the endometriosis and control groups were compared using the McNemar test for paired samples. Pregnancy rates were compared by stage of endometriosis using  $\chi^2$  analysis. All significance tests were done at the  $P < 0.05$  level.

Power analysis demonstrated that this study had 80% power at the 5% significance level to detect a difference of at least 0.5 mm between the endometrial thicknesses of the control group and the endometriosis group. It had 80% power to detect a difference of at least 1 mm between the groups classified by stage of endometriosis. Furthermore, this study had 80% power at the 5% significance level to detect a difference of at least 15% between the pregnancy rates of the control and endometriosis groups and to detect a difference of at least 35% between the groups classified by stage of endometriosis.

## Results

There were 105 patients in each group. The patients with endometriosis were an average ( $\pm$  SD) of  $34.5 \pm 3.5$  years old; the patients without endometriosis were an average of  $34.6 \pm 3.3$  years old. Of the 105 pairs of patients, 66 used the leuprolide acetate/HMG, 29 pairs used the flare and 10

**Table I.** Comparison of IVF parameters according to group. Data presented as mean  $\pm$  SD. No significant differences were found between the means of each group using paired *t*-test.

	Study group: endometriosis ( <i>n</i> = 105)	Study group: no endometriosis ( <i>n</i> = 105)
Serum oestradiol (pg/ml)	1966.5 $\pm$ 1230	1883.3 $\pm$ 1312
Serum progesterone (ng/ml)	0.8 $\pm$ 0.4	0.9 $\pm$ 0.7
No. of follicles $\geq 15$ mm	7.7 $\pm$ 4.8	7.5 $\pm$ 4.9
No. of follicles $\geq 18$ mm	3.7 $\pm$ 3.1	3.2 $\pm$ 2.4
Mean no. of oocytes retrieved	12.6 $\pm$ 8.7	11.8 $\pm$ 6.8
Mean percentage fertilization	63.9 $\pm$ 25.6	66.2 $\pm$ 21.4
Mean no. of embryos transferred	3.7 $\pm$ 1.2	4.1 $\pm$ 1.2
Ovarian stimulation:		
LA/HMG ( <i>n</i> = 66)	40.7 $\pm$ 1.0	40.9 $\pm$ 9.0
Mean no. of ampoules HMG		
Flare ( <i>n</i> = 29)		
Mean no. of ampoules HMG	15.7 $\pm$ 6.11	4.5 $\pm$ 5.4
Mean no. of ampoules FSH	13.4 $\pm$ 9.11	2.9 $\pm$ 8.4
CC <sup>a</sup> /HMG ( <i>n</i> = 10)		
Mean no. of ampoules HMG	15.9 $\pm$ 7.0	15.0 $\pm$ 4.2

LA = leuprolide acetate, HMG = human menopausal gonadotrophin, CC = clomiphene citrate.

<sup>a</sup>CC dosage = 100 mg, days 3–7.

**Table II.** Endometrial characteristics by stage of endometriosis<sup>a</sup>

	Stage I ( <i>n</i> = 20)	Stage II ( <i>n</i> = 37)	Stage III ( <i>n</i> = 26)	Stage IV ( <i>n</i> = 22)
<b>Endometrial thickness (mm)</b>				
Mean $\pm$ SD	12.2 $\pm$ 2.6	13.3 $\pm$ 32.0	12.9 $\pm$ 3.3	12.2 $\pm$ 2.7
Median (mm)	12.0	13.0	12.5	12.0
No. (%) $< 10$ mm	1 (5)	6 (5.4)	2 (7.7)	4 (18.2)
<b>No. with echo pattern (%)</b>				
A	13 (65)	17 (45.9)	13 (50)	13 (59.1)
B	6 (30)	17 (45.9)	13 (50)	8 (36.4)
C	1 (5)	3 (8.1)	0	1 (4.5)
<b>No. of pregnancies (%)</b>				
Clinical 5 (25)	6 (16.2)	7 (26.9)	7 (31.8)	
Ongoing/delivered	5 (25)	6 (16.2)	6 (23.1)	7 (31.8)

<sup>a</sup>No statistically significant differences found by stage.

used the clomiphene citrate/HMG. The average number of ampoules of ovulation-inducing medication was the same in both groups (see Table I).

On the day of HCG administration there was no difference in the mean serum oestradiol concentrations, progesterone concentrations, number of follicles  $\geq 15$  mm or number of follicles  $\geq 18$  mm (see Table I) between the two groups. An average of  $12.6 \pm 8.7$  oocytes were retrieved in the endometriosis group and  $11.8 \pm 6.8$  oocytes in the control group (paired *t*-test, not significant). There was no difference in the fertilization rate or average number of embryos transferred in the two groups (Table I).

The average endometrial thickness in the endometriosis group was  $12.7 \pm 2.9$  mm as compared to  $12.2 \pm 2.5$  mm in the control group (paired *t*-test, not significant). Median endometrial thickness was 12 mm in both groups.

Thin endometrium ( $< 10$  mm) was observed in 12.4% of the endometriosis group and 10.5% of the control group.

**Table III.** Pregnancy rates and endometrial characteristics by infertility factor<sup>a</sup>

	Endometriosis only (n = 50)	Endometriosis and at least one other factor (n = 55)	Tubal factor only (n = 25)	Unexplained infertility (n = 46)	Other (n = 34)
<b>Endometrial thickness (mm)</b>					
Mean $\pm$ SD	12.7 $\pm$ 2.4	12.8 $\pm$ 3.4	11.8 $\pm$ 2.6	12.6 $\pm$ 2.6	12.1 $\pm$ 2.3
Median	12	12	12	12	12
No. <10 mm (%)	6 (12)	4 (7.3)	5 (25)	2 (4.3)	4 (11.8)
<b>No. with echo pattern (%)</b>					
A	25 (50)	31 (56.4)	16 (64.0)	17 (37.0)	19 (55.9)
B	23 (46)	21 (38.2)	7 (28.0)	23 (50)	14 (41.2)
C	2 (4.0)	3 (5.4)	2 (8.0)	6 (13.0)	1 (3.4)
<b>No. of pregnancies (%)</b>					
Clinical	14 (28.0)	11 (20.0)	10 (40.0)	12 (26.1)	14 (41.2)
Ongoing/delivered	13 (26.0)	11 (20)	8 (32.0)	11 (23.9)	11 (32.3)

<sup>a</sup>No significant differences were found by infertility factor.

There was no difference in the distribution of sonographic echo patterns in the two groups ( $\chi^2$ , not significant). In the endometriosis group, pattern A was observed 53.3% of the time, pattern B 41.9% and pattern C 4.8%. In the control group, A was observed 49.5%, B 41.9% and C 8.8% of the time.

There were four abortions in this study, three in the control group (three of 33 pregnancies, 9.1%) and one in the endometriosis group (one of 25 pregnancies, 4%). Abortions were considered as failed clinical pregnancies, i.e. ultrasound evidence of a gestational sac. The patient who aborted in the endometriosis group had stage 3 endometriosis. Thus the abortion rates by stage were 0, 0, 14.3 (one of seven) and 0% respectively.

Three of the aborters had pattern A, one had pattern B. Therefore, the abortion rates by pattern were 7.9% for pattern A, 5% for pattern B and 0% for pattern C.

One of the aborters had endometrial thickness <10 mm and three had thickness  $\geq$ 10 mm. The abortion rates by thickness were 12.5% (one of eight) for those <10 mm and 6.0% (three of 50) for those  $\geq$ 10 mm.

In the endometriosis group, 25 clinical pregnancies (23.8%) were achieved, as compared to 33 (31.4%) in the control group (McNemar, not significant). The ongoing/delivered rates were 22.9 versus 28.6% (McNemar, not significant).

Comparisons of mean endometrial thickness, distribution of echo patterns and pregnancy rates were also made by stage of endometriosis (Table II). There were no statistically significant differences in thickness, pattern or pregnancy rate by stage of endometriosis. Interestingly, pattern C was most associated with reduced pregnancy rates and rarely observed in patients with stage III and IV endometriosis. No reduction in pregnancy rate was observed as the stage of endometriosis became more severe. The clinical pregnancy rate per transfer was 25.0% for stage I, 16.2% for stage II, 26.9% for stage III and 31.8% for stage IV.

There was no difference in the endometrial characteristics of patients with endometriosis as the sole infertility factor and those with endometriosis and other factors (Table III).

When classifying the control group by infertility factor, the average endometrial thickness for patients with tubal factor was  $11.8 \pm 2.6$  mm as compared to  $12.6 \pm 2.5$  mm for

unexplained infertility and  $12.1 \pm 2.3$  mm for other factors. The homogeneous hyperechogenic pattern C was observed in 8% of the tubal patients, 13% of the patients with unexplained infertility and 3% of the patients with other infertility factors. The clinical pregnancy rates per transfer were 40% for tubal factor, 26.1% for unexplained and 41.2% for other factors (Table III).

## Discussion

Based on these data, there is no evidence of a negative effect of endometriosis on the thickness of the endometrium or on its echo pattern as measured on the day of HCG administration. These results are consistent with our findings for women with endometriosis who were followed sonographically in the cycle before and after laparoscopic treatment for endometriosis (Check *et al.*, 1993a). In that study, the mean endometrial thickness was the same for patients with endometriosis and the control group both before and after surgical treatment for endometriosis.

Several studies (Morcos *et al.*, 1985; Prough *et al.*, 1990; Abu-Musa *et al.*, 1992a;b) have reported the presence of an embryotoxic factor in the peritoneal fluid of women with endometriosis; this finding suggested the possibility that toxic cytokines might interfere with implantation (and that this would be reflected by thin endometria or certain sonographic echo patterns). However, the data presented herein, showing no reduction in pregnancy rates or increase in spontaneous abortion rates following embryo transfer compared to controls, even in cases of severe endometriosis (stages 3 and 4), strongly suggest that if toxic cytokines are involved in the aetiology of reduced pregnancy rates, the mechanism does not involve impaired implantation.

## References

- Abu-Musa, A., Takahashi, K. and Kitao, M. (1992a) The effect of serum obtained before and after treatment for endometriosis on the in vitro development of two-cell mouse embryos. *Fertil. Steril.*, **57**, 1098-1102.
- Abu-Musa, A., Takahashi, K. and Manubu, K. (1992b) Effect of serum from patients with endometriosis on the development of mouse embryos. *Gynecol. Obstet. Invest.*, **33**, 157-160.

- American Fertility Society. (1985) Revised American Fertility Society classification of endometriosis. *Fertil. Steril.*, **43**, 351-352.
- Check, J.H., Nowroozi, K., Choe, J. and Dietterich, C. (1991) Influence of endometrial thickness and echo patterns on pregnancy rates during in vitro fertilization. *Fertil. Steril.*, **56**, 1173-1175.
- Check, J.H., Dietterich, C., Lurie, D. and Adelson, H.G. (1993a) The relationship of endometrial thickness and echo pattern to endometriosis. 9th Annual Meeting of the European Society of Human Reproduction and Embryology. *Hum. Reprod.*, **8**, (Suppl. 1), 163.
- Check, J.H., Lurie, D., Dietterich, C., Callan, C. and Baker, A. (1993b) Adverse effect of a homogeneous hyperechogenic endometrial sonographic pattern despite adequate endometrial thickness of pregnancy rates following in-vitro fertilization. *Hum. Reprod.*, **8**, 1293-1296.
- Check, J.H., Nowroozi, K., Choe, J., Lurie, D. and Dietterich, C. (1993c) The effect of endometrial thickness and echo pattern on in-vitro fertilization outcome in donor oocyte-embryo transfer cycles. *Fertil. Steril.*, **59**, 72-75.
- Garcia, J.E., Padilla, S.L., Bayati, J. and Baramki, T.A. (1990) Follicular phase gonadotropin-releasing hormone agonist and human gonadotropins: a better alternative for ovulation induction in in vitro fertilization. *Fertil. Steril.*, **53**, 302-305.
- Gonen, Y. and Casper, R.F. (1990) Prediction of implantation by the sonographic appearance of the endometrium during controlled ovarian stimulation for in vitro fertilization (IVF). *J. In Vitro Fertil. Embryo Transfer*, **7**, 146-152.
- Gonen, Y., Casper, R.F., Jacobson, W. and Blankier, J. (1989) Endometrial thickness and growth during ovarian stimulation: a possible predictor of implantation in in-vitro fertilization. *Fertil. Steril.*, **52**, 446-450.
- Khalifa, E., Brzyski, R.G., Oehninger, S., Acost, A.A. and Muasher, S.J. (1992) Sonographic appearance of the endometrium; the predictive value for the outcome of in-vitro fertilization in stimulated cycles. *Hum. Reprod.*, **7**, 677-680.
- Matson, P.L. and Yovich, J.L. (1986) The treatment of infertility associated with endometriosis by in vitro fertilization. *Fertil. Steril.*, **46**, 432-434.
- Meldrum, D.R., Wisot, A., Hamilton, F., Gutlay, A.L., Kempton, W. and Huynh, D. (1989) Routine pituitary suppression with leuprolide before ovarian stimulation for oocyte retrieval. *Fertil. Steril.*, **51**, 455-459.
- Morcos, R.N., Gibbons, W.E. and Findley, W.E. (1985) Effect of peritoneal fluid on in vitro cleavage of 2-cell mouse embryos: possible role in infertility associated with endometriosis. *Fertil. Steril.*, **44**, 678-683.
- Oehninger, S., Acosta, A.A., Kreiner, D., Muasher, S.J., Jones, H.W. and Rosenwaks, Z. (1988) In vitro fertilization and embryo transfer (IVF/ET): an established and successful therapy for endometriosis. *J. In Vitro Fertil. Embryo Transfer*, **5**, 249-256.
- Prough, S.G., Aksel, S., Gilmore, S.M. and Yeoman, R.R. (1990) Peritoneal fluid from patients with endometriosis does not promote two cell mouse embryo growth. *Fertil. Steril.*, **54**, 927-930.
- Smith, B., Porter, R., Ahuja, K. and Craft, I. (1984) Ultrasonic assessment of endometrial changes in stimulated cycles in an in vitro fertilization and embryo transfer program. *J. In Vitro Fertil. Embryo Transfer*, **1**, 233-238.
- Wardle, P.G., Foster, P.A., Mitchell, J.D., McLaughlin, E.A., Sykes, J.A.C., Corrigan, E., Hull, M.G.R., Ray, B.D. and McDermott, A. (1986) Endometriosis and IVF: effect of prior therapy. *Lancet*, **i**, 267-276.
- Yovich, J.L., Matson, P.L., Richardson, P.A. and Hilliard, C. (1988) Hormonal profiles and embryo quality in women with severe endometriosis treated by in vitro fertilization and embryo transfer. *Fertil. Steril.*, **50**, 308-313.