

Adverse effect of a homogeneous hyperechogenic endometrial sonographic pattern, despite adequate endometrial thickness on pregnancy rates following in-vitro fertilization

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We have previously presented data to show that in patients who had in-vitro fertilization (IVF)–embryo transfer using ovarian stimulation involving the luteal phase leuprolide acetate–human menopausal gonadotrophin (HMG) regimen, poor pregnancy results ensued if either the endometrial thickness was <10 mm or a homogeneous hyperechogenic sonographic pattern was present immediately prior to taking a human chorionic gonadotrophin (HCG) injection. There were only 15 cases with this hyperechogenic type endometrium (and no pregnancies). The purpose of the present study was to evaluate the influence of a hyperechogenic endometrium when the endometrial thickness was ≥ 10 mm, in a more extensive series, in women having IVF–embryo transfer using the same ovarian stimulation regimen. A total of 273 consecutive cycles, where endometrial thickness was ≥ 10 mm, were evaluated (not including the 85 cycles previously reported). Of 22 patients with the hyperechogenic pattern, one achieved a chemical pregnancy (β -HCG >500 mIU/ml) and none achieved clinical pregnancies (ultrasound confirmation). In contrast, 67 of 251 (26.7%) patients conceived with other echo patterns (χ^2 analysis = 5.9, df = 1, $P = 0.01$). These data thus confirm, in a larger series, the negative influence of this type of echo pattern on subsequent pregnancy rates following the luteal phase leuprolide acetate–HMG ovarian stimulation regimen.

Key words: endometrial echo pattern/homogeneous/hyperechogenic/in-vitro fertilization

Introduction

Vaginal sonography to detect changes in endometrial thickness and texture, prior to the rise in progesterone, has been used to predict the likelihood of pregnancy. Although one study of endometrial thickness in in-vitro fertilization (IVF)–embryo transfer cycles found no correlation between endometrial thickness and pregnancy rates (Fleischer *et al.*, 1986), other studies have shown that when the endometrium is too thin the

pregnancy rates suffer (Gonen *et al.*, 1989; Dickey *et al.*, 1992). Even with non-IVF cycles, increased thickness has correlated with better pregnancy rates (Shoham *et al.*, 1991).

Endometrial echo patterns are determined by the comparison of the echogenicity of the endometrium to that of the myometrium and the distinction of a central echogenic line within the endometrium. These echo patterns have been described as appearing either hypo-echogenic with well-defined outer walls and central echogenic line, iso-echogenic with a poorly defined central echogenic line, or hyperechogenic with no visualization of the central echogenic line (Smith *et al.*, 1984; Gonen and Casper, 1990). The comparison of these echo patterns seen prior to oocyte recovery with subsequent pregnancy rates has shown varying results in different studies of women undergoing ovarian stimulation for IVF–embryo transfer. Gonen *et al.* (1990) found that only the hypo-echogenic endometrial echo pattern correlated with good pregnancy rates when the ovarian stimulation regimen was clomiphene citrate–human menopausal gonadotrophin (HMG) and the sonographic evaluation was performed on the day following the injection of human chorionic gonadotrophin (HCG), which was 1 day prior to oocyte recovery.

In a study by Sher *et al.* (1991) higher pregnancy rates were also demonstrated when a thicker hypo-echogenic endometrium was seen on the day of HCG injection. This study compared only the hypo-echogenic to the hyperechogenic echo pattern and the ovarian stimulation regimen was luteal leuprolide acetate, which was combined with HMG and follicle stimulating hormone (FSH) once adequate gonadotrophin suppression was achieved.

Check *et al.* (1991), however, published data showing that only the hyperechogenic pattern correlated with a poor pregnancy rate in comparison to the hypo-echogenic and iso-echogenic patterns seen on the day of HCG injection when the ovarian stimulation regimen was luteal leuprolide acetate combined with HMG. This study included only 15 cases with a hyperechogenic pattern where not all had a pre-ovulatory endometrial thickness of ≥ 10 mm (this thickness was found to distinguish conception from non-conception cycles).

The importance of increased endometrial thickness and IVF outcome has also been found in donor-oocyte transfer cycles. A separate study demonstrated a positive correlation between the endometrial thickness of donor-oocyte recipients who received oestrogen replacement therapy and subsequent pregnancy rates. Sonographic evaluation of endometrial thickness and echo pattern of the recipient was performed on the day of the donor's HCG injection, which was prior to the start of progesterone replacement in the recipient. There was, however, no difference in pregnancy rates according to echo pattern (Check *et al.*, 1993).

The study presented here was designed to re-evaluate the

correlation of endometrial echo patterns using the luteal leuprolide acetate combined with ovarian stimulation regimen when the endometrial thickness reached a minimum of 10 mm in a larger series.

Materials and methods

All IVF-embryo transfer cycles from September 1991 to July 1992 were evaluated in which the luteal phase leuprolide acetate-HMG regimen for ovarian stimulation was used (Meldrum *et al.*, 1989). Each patient started leuprolide acetate 1 mg, on day 21 of the cycle. After 10 days of leuprolide acetate, serum oestradiol, progesterone and luteinizing hormone (LH) concentrations were obtained to ascertain whether adequate gonadotrophin suppression had been achieved (oestradiol <30 pg/ml, progesterone <1.0 ng/ml), and a sonogram was performed to rule out ovarian cysts. If either the serum concentrations or sonogram were inadequate, 1 mg leuprolide acetate was continued until the desired results were attained. Then the dosage of leuprolide acetate was reduced to 0.5 mg and HMG (300 IU/day) was added. Vaginal sonography and serum oestradiol, progesterone and LH concentrations were obtained daily after 5 days of leuprolide acetate-HMG. Changes in dosage of HMG were made, if necessary, according to serum oestradiol concentration and size of follicles. HCG, 10 000 IU, was administered when a minimum of two lead follicles with an 18 mm average diameter were seen sonographically and the serum oestradiol was at least 300 pg/ml per follicle; HCG was delayed and leuprolide acetate-HMG was continued for 1-2 days even if these criteria were met, if the endometrial thickness was <10 mm. After 2 days, if the endometrial thickness was still <10 mm, HCG was given, oocyte recovery occurred and the patient was advised to have all embryos cryopreserved.

Endometrial sonographic evaluation was performed using an ATL Ultramark 4 (Advanced Technology Laboratories, Bothell, WA, USA) equipped with a 5 MHz endovaginal transducer. Thickness was measured in mm by placing electronic calipers on the outer walls of the endometrium as seen in the longitudinal axis of the uterine body. The endometrial echo patterns were graded I, II, or III using the following criteria: pattern I represented the hypo-echogenic endometrium with well-defined outer walls and central echogenic line; pattern II was used to represent the iso-echogenic endometrium with a poorly defined central echogenic line; pattern III was used to denote the homogeneous hyperechogenic endometrium in which no central echogenic line was visualized (Grunfeld *et al.*, 1991). All measurements and grading were performed by one experienced sonographer on one ultrasound unit to eliminate inter-observer and machine variation.

Endometrial thickness and echo pattern from the day of HCG injection were recorded. A total of 307 consecutive cycles was available for evaluation. Only cycles demonstrating an endometrial thickness of ≥ 10 mm were included, thus eliminating 16 cycles. No fertilization was achieved in 18 cycles, making 273 cycles available for complete evaluation. No inclusion or exclusion was made based on echo pattern.

Parameters relevant to the IVF cycle, including patient's age, serum oestradiol and progesterone concentrations on day of HCG

injection, number of oocytes retrieved, number of follicles observed, number of embryos transferred, and conception outcome were also recorded for each cycle. The oestradiol was measured by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA) and measurement of serum progesterone by radioimmunoassay (Amersham Corporation, Arlington Heights, IL, USA). The pregnancy rates were compared according to the three different echo patterns (in all cases thickness was ≥ 10 mm). A clinical pregnancy was established only with ultrasound evidence of pregnancy; a viable pregnancy was defined as a pregnancy showing sonographic viability 6 weeks from retrieval.

Statistical methods

The pregnancy rates observed were compared by echo pattern using χ^2 -analysis. Significant differences were further analysed by partitioning the χ^2 . Comparisons between the echo pattern groups and other IVF parameters were made using analysis of variance (ANOVA). If the data on a variable were not normally distributed, the Kruskal-Wallis ANOVA was used. All testing was done at the 5% level of significance.

Results

There were 273 cycles in which the patient's endometrium attained a thickness of at least 10 mm at the time of HCG injection.

In these cycles, 151 (55% had echo pattern I, 100 (37%) had echo pattern II and 22 (8%) had echo pattern III. The distribution of echo pattern for specific indication for IVF is presented in Table I. Pattern I occurred in between 52 and 60% of all indications, pattern II between 28 and 40% with the highest for tubal and lowest for unexplained infertility, while pattern III occurred between 6% and 14% of all cycles, with the highest in the group with ovarian dysfunction/polycystic ovarian disease (PCO) and the lowest in the groups with male factor and endometriosis. The distributions of echo patterns were not significantly different by infertility indication ($\chi^2 = 3.5$, $P = 0.895$).

Table I. Distribution of echo pattern by indication for in-vitro fertilization (IVF)^a

Indication for IVF	Echo pattern I (%)	Echo pattern II (%)	Echo pattern III (%)
Tubal	74 (52)	56 (40)	11 (8)
Unexplained	18 (62)	8 (28)	3 (10)
Endometriosis	19 (56)	13 (38)	2 (6)
Male factor	29 (60)	16 (33)	3 (6)
Miscellaneous (ovarian dysfunction, PCO, cervical)	11 (52)	7 (33)	3 (14)

^a $\chi^2 = 3.5$, $df = 8$, $P = 0.895$.

Pattern I = hypo-echogenic endometrium, well-defined outer walls, central echogenic line.

Pattern II = iso-echogenic endometrium, poorly defined central echogenic line.

Pattern III = homogeneous hyperechogenic endometrium, no central echogenic line.

PCO = polycystic ovarian disease.

There was no difference in the mean age of the patients with each pattern type: 33.9 ± 3.5 years for pattern I, 33.9 ± 3.4 years for pattern II and 31.8 ± 4.0 years for pattern III (ANOVA, $P = 0.06$). Day 13 of the cycle was the median day in all three echo pattern groups when HCG was administered.

Comparison of the serum hormone concentrations of oestradiol and progesterone on the day of HCG by echo pattern showed that there was no difference in the median concentrations observed by echo pattern (1906 pg/ml and 0.9 ng/ml respectively for I, 1748 pg/ml and 0.9 ng/ml respectively for II and 2060 pg/ml and 1.0 ng/ml respectively for III; Kruskal-Wallis ANOVA, $P = 0.293, 0.345$). The distribution of echo pattern by progesterone and oestradiol concentration is presented in Table

Table II. Comparison of distribution of echo patterns by serum progesterone and oestradiol concentrations on day of human chorionic gonadotrophin (HCG)^a

	Pattern I ^c No. (%)	Pattern II ^c No. (%)	Pattern III ^c No. (%)
Progesterone (ng/ml)			
≤ 1.0	86 (53.1)	65 (40.1)	11 (6.8)
1.1–1.5	48 (60.8)	25 (31.6)	6 (7.6)
> 1.5	17 (53.1)	10 (31.3)	5 (15.6)
Oestradiol ^b (pg/ml)			
< 1000	26 (65.0)	13 (32.5)	1 (2.5)
1001–2000	54 (51.9)	42 (40.4)	8 (7.7)
2001–3000	41 (58.6)	21 (30.0)	8 (11.4)
3001–4000	18 (62.1)	10 (34.5)	1 (3.4)
> 4000	12 (40.0)	14 (46.7)	4 (13.3)

^a $\chi^2 = 4.5$, $df = 4$, $P = 0.337$.

^b $\chi^2 = 9.2$, $df = 8$, $P = 0.284$.

^cSee Table I footnote.

Table III. Pregnancy rates by echo pattern^a

	Pattern I No. (%)	Pattern II No. (%)	Pattern III No. (%)
Overall ^b	44/151 (29.1)	23/100 (23.0)	1/22 (4.5)
Clinical ^c	37/151 (24.5)	17/100 (17.0)	0/22 (0.0)
Chemical ^d	7/151 (4.6)	6/100 (6.0)	1/22 (4.5)

^aSee footnote to Table I.

^b $\chi^2 = 6.5$, $P = 0.038$ comparing overall pregnancy rates by echo pattern.

Partitioning the χ^2 , $\chi^2 = 1.16$, $P = 0.282$ comparing overall rates for I versus II; $\chi^2 = 5.3$, $P = 0.021$ comparing rates for I and II combined versus III.

^c $\chi^2 = 8.03$, $df = 2$, $P = 0.018$ comparing pregnancy rate by echo pattern;

$\chi^2 = 2.0$, $P = 0.157$, comparing pregnancy rate of pattern I versus II;

$\chi^2 = 5.9$, $P = 0.015$, comparing pregnancy rate for I and II combined versus III.

^d $\chi^2 = 0.247$, $P = 0.884$ comparing chemical pregnancy rates by echo

pattern.

Table IV. Comparisons of factors related to in-vitro fertilization (IVF) outcome by echo pattern

	Pattern I ^a ($n = 151$)	Pattern II ^a ($n = 100$)	Pattern III ^a ($n = 22$)	P^b
Median no. of oocytes retrieved	12.0	12.0	16.0	0.170
Median no. of embryos transferred	5.0	4.0	4.5	0.479
Median % fertilization	69.0	67.0	69.0	0.935

^aSee Table I footnote.

^bBased on Kruskal-Wallis analysis of variance.

II. The distribution of echo patterns was the same for all progesterone concentrations ($\chi^2 = 4.5$, $P = 0.337$, Table II). However, it is interesting to note that once progesterone concentration rose above 1.5 ng/ml, the proportion of pattern III endometrium observed increased from 6.8 to 15.6%. The distribution of echo patterns at each oestradiol concentration was the same ($\chi^2 = 9.2$, $df = 8$, $P = 0.284$).

There was a significant difference in the endometrial thickness by echo pattern with the median of 12.0 mm for pattern I, 13.0 mm for pattern II and 14.0 mm for pattern III (Kruskal-Wallis, $P = 0.002$).

There was a total of 68 (24.9%) pregnancies observed, 54 (19.8%) clinical and 14 (5.1%) chemical (β -HCG concentration > 500 mIU/ml but no ultrasound confirmation). The pregnancy rates by echo pattern are presented in Table III. The clinical pregnancy rate was 24.5% for pattern I, 17.0% for pattern II and 0% for pattern III. χ^2 -analysis showed that there was a significant difference in the clinical pregnancy rates by echo pattern (χ^2 , $P = 0.01$). Further comparisons were made by partitioning the χ^2 results. These comparisons showed that there was no difference in the rates for patterns I and II (χ^2 , $P = 0.16$); however, when comparing the rates for patterns I and II combined against that for pattern III, there was a significant difference in the pregnancy rates (χ^2 , $P = 0.02$).

Other possible factors that could contribute to the conception outcome of the IVF cycle were compared by echo pattern to see if the groups differed in these parameters. However, there was no difference in the median number of oocytes retrieved, fertilization rate, or number of embryos transferred (Table IV).

Discussion

Previously no pregnancies were found in any patients with a type III pattern (Check *et al.*, 1991). Similarly, in this study there were no clinical pregnancies, though there was one chemical pregnancy. The previous failure to determine any significant difference between patterns I and II was once again demonstrated in the present study.

The different echo patterns have been reported to coincide with the changes of the endometrium in response to oestradiol and progesterone concentrations throughout the menstrual cycle. Hyperechogenic endometrium (III) is the pattern typically seen in the mid- to late secretory phase (Grinfeld *et al.*, 1991). We have also observed that the pattern becomes hyperechogenic once the progesterone concentration rises. Previous data from our clinic shows that there is a lower pregnancy rate if the progesterone concentration rises above 1 ng/ml prior to the HCG injection (Check *et al.*, 1992). Others have drawn the same conclusions

(Schoolcraft *et al.*, 1991; Silverberg *et al.*, 1991). We hypothesized that perhaps those with pattern III might have higher serum progesterone concentration prior to HCG injection; however, no statistical differences were found amongst the three groups, though pattern III had the highest median (1.0). The reason for observing this echo pattern pre-ovulatory is not known.

Though the percentage of patients with pattern III was more than twice as high when the serum progesterone was ≥ 1.6 ng/ml than when it was ≤ 1 ng/ml (5/32 or 15.6% versus 11/162 or 6.8%), more than half of those patients with serum progesterone > 2 ng/ml still had type I patterns. Thus, our data do not support the hypothesis that higher serum progesterone concentrations are responsible for the pre-ovulatory observation of pattern III, and could thus explain the poor pregnancy rate by over-advancement of the endometrium by excessive progesterone.

Though the exact mechanism is not known, at this time our data would suggest that if a patient has met the normal follicular maturation criteria to give HCG in an IVF programme, if a type III endometrium is seen cryopreservation should be considered. Whether pushing the stimulation further can change the echo pattern remains to be determined by further studies.

References

- 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., Choe, J., Nowroozi, K. and Hoover, L. (1992) The influence of the serum progesterone level at the time of hCG on subsequent pregnancy rates from in vitro fertilization–embryo transfer (IVF–ET). Presented at the 48th Annual Meeting of the American Fertility Society, November 1992. Abstr. No. P-056, p. S136.
- Check, J.H., Nowroozi, K., Choe, J., Lurie, D. and Dietterich, C. (1993) The effect of endometrial thickness and echo pattern on in vitro fertilization outcome in donor oocyte–embryo transfer cycle. *Fertil. Steril.*, **59**, 72–75.
- Dickey, R.P., Olar, T.T., Curole, D.N., Taylor, S.N. and Rye, P.H. (1992) Endometrial pattern and thickness associated with pregnancy outcome after assisted reproduction technologies. *Hum. Reprod.*, **7**, 418–421.
- Fleischer, A.C., Herbert, C.M., Sacks, G.A., Wentz, A.C., Entman, S.S. and James, A.E., Jr (1986) Sonography of the endometrium during conception and nonconception cycles of in vitro fertilization and embryo transfer. *Fertil. Steril.*, **46**, 442–447.
- Gonen, Y. and Casper, R.F. (1990) Prediction of implantation by sonographic appearance of 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.
- Grunfeld, L., Walker, B., Bergh, P.A., Sandler, B., Hofmann, G. and Navot, D. (1991) High-resolution endovaginal ultrasonography of the endometrium: A noninvasive test for endometrial adequacy. *Obstet. Gynecol.*, **78**, 200–204.
- 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.
- Schoolcraft, W., Sinton, E., Schlenker, T., Huynh, D., Hamilton, F. and Meldrum, D.R. (1991) Lower pregnancy rate with premature luteinization during pituitary suppression with leuprolide acetate. *Fertil. Steril.*, **55**, 563–566.

Sher, G., Herbert, C., Maassarani, G. and Jacobs, M.H. (1991) Assessment of the late proliferative phase endometrium by ultrasonography in patients undergoing in-vitro fertilization and embryo transfer (IVF/ET). *Hum. Reprod.*, **6**, 232–237.

Shoham, Z., Di Carlo, C., Patel, A., Conway, G.S. and Jacobs, H.S. (1991) Is it possible to run a successful ovulation induction program based solely on ultrasound monitoring? The importance of endometrial measurements. *Fertil. Steril.*, **56**, 836–841.

Silverberg, K.M., Burns, W.N., Olive, D.L., Riehl, R.M. and Schenkens, R.S. (1991) Serum progesterone levels predict success of in vitro fertilization/embryo transfer in patients stimulated with leuprolide acetate and human menopausal gonadotropins. *J. Clin. Endocrinol. Metab.*, **73**, 797–803.

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.

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