

Non-homogeneous hyperechogenic echo pattern three days after frozen embryo transfer is associated with lower pregnancy rates

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Summary

Objective: To evaluate the association of mid-luteal phase echo patterns and pregnancy rates (PRs) following frozen embryo transfer (ET).

Methods: Sonographic evaluation of endometrial echo patterns was performed three days after ET in the first frozen ET cycle of women < 40 years of age who used their own oocytes as well as all donor oocyte recipients.

Results: The distribution of echo patterns and clinical PRs were similar in women using their own eggs and women who used donor oocytes; therefore all data was combined. The clinical PR was 49.5% with a hyperechogenic echo pattern vs 38.8% with a non-hyperechogenic pattern, $p = .007$.

Conclusion: A larger study of frozen ET and mid-luteal echo pattern now demonstrates conclusions similar to the data from fresh ET in hyperstimulated in vitro fertilization (IVF)-ET cycles in that failure to attain a hyperechogenic echo pattern three days after ET is associated with lower pregnancy rates.

Key words: Frozen embryo transfer; Endometrial echo patterns; Mid-luteal sonography.

Introduction

Sonography has been utilized as a non-invasive technique to monitor ovulation for over two decades. Sonographic changes in the appearance of the endometrial echo pattern throughout the menstrual cycle have been observed [1-5]. Three echo patterns have been described as triple-line (TL), isoechogenic (IE), and homogeneous hyperechogenic (HH). Most of the sonographic evaluations of endometrial echo pattern have focused on the time prior to or on the day of embryo transfer (ET). At this time the endometrium should demonstrate a good estrogen effect, represented sonographically as a TL echo pattern. In the secretory phase, the endometrium should have an HH texture with no presence of a central echogenic line and is more echogenic than the surrounding myometrium. This appearance is most likely due to rising progesterone (P) levels.

Some researchers have found that the absence of an HH pattern in the luteal phase is an indication for further evaluation [6, 7]. In one previously published study, it was found that the mid-luteal phase appearance of the endometrium affected in vitro fertilization (IVF)-ET outcome in fresh ET cycles following oocyte retrieval [8]. In the same study no association was found between the mid-luteal echo pattern and outcome in frozen ET cycles; however, the conclusions were impeded by relatively small study samples [8]. More recently, one study reported decreased fecundity in women with a non-HH echo pattern

in the mid-luteal phase who were not treated with follicle maturing drugs and IVF [9].

The objective of the current study was to evaluate the association of mid-luteal phase echo patterns three days after ET and outcome in a larger series of patients. The study was designed to include the first frozen ET cycle of women < 40 years of age who used their own oocytes as well as all donor oocyte recipients.

Materials and Methods

A prospective observational study was performed to evaluate frozen ET cycles from 1/1/97 to 4/30/02 of all women who were < 40 years of age at the time of oocyte retrieval and all donor oocyte recipients. During the study period, laboratory conditions and protocols were uniform. All patients underwent uterine cavity evaluation by hysterosalpingogram, hysteroscopy, or saline infusion sonography demonstrating a normal uterine cavity within two years prior to ET. Patients had to have had sonographic monitoring three days after ET in our facility to be included in the study. Only cycles using estrogen (E2)/progesterone (P) replacement, either with or without down-regulation were used in the analysis. Cycles were stratified by the source of oocytes, i.e., women who used their own oocytes versus donor egg recipients.

Preparation for frozen ET included down-regulation with leuprolide acetate (LA) and E2/P replacement or E2/P replacement alone. Estrogen replacement in the form of oral micronized E2 was administered in graduating dosages beginning on day 2 of their cycle; i.e., 2 mg x five days followed by 4 mg x four days and 6 mg x five days. If down regulation was utilized, LA was administered ten days prior to the expected menses and continued until the oral E2 dosage was increased to 4 mg. Patients given medications, such as baby aspirin, heparin, estrogen tablets inserted vaginally or sildenafil, to improve mid-

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cycle endometrial thickness were excluded from the study. Progesterone supplementation was added to oral E2 when an endometrial thickness of at least 8 mm with a trilaminar echo pattern was observed sonographically.

All ETs were performed on the fourth day of P supplementation. The cryopreservation technique used was a simplified method in which a slow cooling program is started at the seeding temperature of -6°C in an alcohol bath controlled rate freezer; 1,2 propanediol was used as the cryoprotectant. A one-step fast thawing procedure at room temperature was used and the cryoprotectant was removed from the embryos in one step [10].

Transvaginal sonogram and sera hormone levels of E2 and P4 were first performed on day 14 or 15 of E2 and continued up until day 20 if there was an initial inadequate endometrial response. Mid-luteal sonographic assessment and sera hormonal levels of E2 and P4 were obtained three days after ET in all women.

Real time sonographic assessment included measurements of endometrial thickness, grading of the endometrial echo pattern and color Doppler analysis of the uterine arteries. Endometrial thickness was measured by placing electronic calipers on the outer walls of the endometrium at the widest diameter seen in the longitudinal axis. Endometrial echo patterns were graded as TL, IE, or HH. For the purpose of the analysis, the mid-luteal echo pattern was classified as HH vs non-HH.

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. 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 for each measurement was used in the analysis.

All sonograms were performed on a GE Logic 400 (General Electric Medical Systems, Milwaukee, WI). To eliminate inter-observer variability, all endometrial grading and calculations of the PI and RI were performed by one sonographer. The sonographer was blinded as to the time of the cycle (pre or post ET) that the sonograms were performed.

Only the first cycle per patient was used to assure independence of observations. The main outcome measures were clinical pregnancy (sonographic confirmation of a gestational sac in the uterus), ongoing pregnancy (live pregnancy after the first trimester) and implantation rates (number of gestational sacs/embryos transferred). Other variables considered were age of the patient, number of days of E2 prior to start of P, number of ET, serum E2 and P4 levels, Doppler indices of PI and RI, and endometrial thickness both prior to start of P therapy and in the mid-luteal phase. Statistical methodologies of chi-square analysis and independent t-tests were used as appropriate. A p value of .05 was used to determine significance.

Results

The first frozen ET cycle was evaluated in 671 women using their own oocytes and 188 women who used donor oocytes. The distribution of echo patterns (HH vs non-HH) were similar despite the source of oocytes; 74.2% of women using their own oocytes and 76.6% of oocyte recipients demonstrated an HH echo pattern three days after ET. Overall clinical PRs also did not differ between the two groups (47.5% vs 43.1%, respectively); therefore data from all patients were combined for the analysis.

Clinical and ongoing PRs as well as implantation rates were all significantly higher when a HH echo pattern was observed three days after ET (Table 1). The multiple PR by echo pattern showed no difference; 38.7% (122/315) when the HH pattern was seen vs 31.4% (27/86) when it was not observed. Spontaneous abortion rate also did not differ between the two groups; 17.4% vs. 16.3%, respectively.

Table 1. — Comparison of frozen embryo transfer outcome by endometrial echo pattern three days after embryo transfer.

	Homogeneous hyperechogenic pattern observed (n = 637)	Homogeneous hyperechogenic pattern not observed (n = 222)	p value
Clinical pregnancy	49.5% (315/637)	38.8% (86/222)	.007
Ongoing pregnancy rate	41.1% (262/637)	31.5% (72/222)	.014
Implantation rate	21.4% (457/2133)	16.9% (117/691)	.013

Contributing factors, such as age, number of days taking E2 prior to starting P, number of ET, sera hormonal levels or other sonographic parameters, did not differ by echo pattern three days after ET (Table 2). There was no threshold in which a clinical pregnancy or spontaneous abortion could be identified.

Table 2. — Comparison of contributing factors by endometrial echo pattern three days after embryo transfer (data presented as mean \pm SD)*.

	Homogeneous hyperechogenic pattern (n = 637)	Non-homogeneous hyperechogenic pattern (n = 222)
Age (years)	35 \pm 6.0	35.4 \pm 5.5
Number of days of E2	15.7 \pm 1.70	15.3 \pm 1.92
Number of embryos transferred	3.38 \pm 1.04	3.10 \pm .98
<i>Sera hormonal levels</i>		
<i>Mid cycle</i>		
E2 (pg/ml)	1149.57 \pm 740.5	1188.54 \pm 792.5
P4 (ng/ml)	.63 \pm .56	.60 \pm .56
<i>Mid-luteal</i>		
E2 (pg/ml)	995.41 \pm 520.11	1001.48 \pm 540.04
P4 (pg/ml)	56.9 \pm 31.0	55.67 \pm 28.23
<i>Sonographic parameters</i>		
<i>Endometrial thickness</i>		
Mid cycle	10.12 \pm 1.28	10.13 \pm 1.28
Mid-luteal	10.25 \pm 2.5	10.79 \pm 2.52
<i>Pulsatility index</i>		
Mid cycle	2.66 \pm .72	2.70 \pm .76
Mid-luteal	2.79 \pm .75	2.87 \pm .71
<i>Resistance index</i>		
Mid cycle	.88 \pm .06	.89 \pm .06
Mid-luteal	.89 \pm .06	.89 \pm .05

*P = NS.

We then sought to determine if any factors were different between women who achieved a clinical pregnancy vs those who did not. Comparison of all factors by outcome revealed no differences with the exception of a higher distribution of the HH pattern and lower distribution of trilaminar echo patterns in women who achieved a clinical pregnancy vs those who failed to achieve a pregnancy (Table 3).

Table 3. — Comparison of factors by outcome.

	Clinical pregnancy achieved (n = 401)	No pregnancy achieved (n = 458)
Age (years) ^a	35.0 ± 6.0	35.5 ± 5.5
Number of days of E2	15.34 ± 1.83	15.34 ± 1.84
Number of embryos transferred	3.43 ± 0.91	3.16 ± 1.0
<i>Sera hormonal levels</i>		
<i>Mid cycle</i>		
E2 (pg/ml) ^a	1163.99 ± 672.33	1160.94 ± 597.54
P4 (ng/ml) ^a	.60 ± .52	.56 ± .51
<i>Mid luteal</i>		
E2 (pg/ml) ^a	995.55 ± 505.94	1001.16 ± 501.99
P4 (pg/ml) ^a	55.5 ± 30.0	57.95 ± 30.55
<i>Sonographic parameters</i>		
<i>Endometrial thickness</i>		
Mid cycle ^a	10.43 ± 1.83	10.34 ± 1.98
Mid luteal ^a	10.43 ± 2.50	10.34 ± 2.45
<i>Pulsatility index</i>		
Mid cycle ^a	2.68 ± .73	2.65 ± .73
Mid luteal ^a	2.81 ± .72	2.81 ± .75
<i>Resistance index</i>		
Mid cycle ^a	.88 ± .06	.88 ± .06
Mid luteal ^a	.88 ± .05	.89 ± .05
<i>Echo pattern 3 days after</i>		
<i>Embryo transfer</i>		
Trilaminar ^b	21.4% (86/401)	29.7% (136/458)
Hyperechogenic ^b	78.6% (315/401)	70.3% (322/458)

^aP = NS, independent t-test, data presented as mean ± SD.
^bP = .007, Chi square.

Discussion

Embryo quality and uterine environment have been demonstrated to be the most critical factors in predicting successful IVF-ET outcome. The majority of the studies of endometrial thickness and echo pattern have focused on these parameters prior to or at the time of ET. With improved PRs over the last decade, most studies now conclude that endometrial thickness prior to fresh ET [11-18] or frozen ET [19] has only marginal prognostic value when only the extremes of poor growth are seen. However, it has been well established that a trilaminar echo pattern prior to oocyte retrieval is an important factor in achieving a positive outcome in IVF-ET [11-13, 20].

Similarly, endometrial thickness after frozen ET does not correlate with PRs, as seen in the present study as well as in an earlier investigation [8]. Although all women had an endometrial thickness ≥ 8 mm prior to ET, 9.0% (36/401) of women who achieved a pregnancy and 9.4% (43/458) of women who failed to become pregnant had a thickness < 8 mm three days after ET. Thinner endometria did not correlate with poorer echo pattern; in women with mid-luteal phase endometrial thickness < 8 mm six of 36 (16.7%) of the pregnant group and seven of 43 (16.3%) of the non-pregnant group failed to convert to the HH pattern which was less than observed for all women in each group.

The mechanism for failure of the endometrium to change from the triple line pattern to homogeneous hyperechogenic is unknown. Serum E2 and P levels cannot always accurately predict endometrial development [21, 22]. No relationship between echo pattern and

serum P or E2 levels was observed. The lowest serum P level was 11.2 ng/ml when the HH pattern was seen and 8.5 ng/ml when a trilaminar pattern was seen; the lowest serum P level where a clinical pregnancy was seen was 11.5 ng/ml. Two pregnancies were seen at this level, one with the HH pattern and the other with the TL pattern and both pregnancies delivered. Possibly, future studies will determine if there is a relationship between luteal echo pattern and endometrial E2 and P receptors or their ratio. One previously reported study did not find any relationship between pre-ovulatory echo pattern and endometrial steroid hormone receptor concentration, but the ratio of P:E2 concentration was slightly lower when a pre-ovulatory TL echo pattern was not seen [23].

Endometrial biopsies in IVF cycles have indicated that ovarian stimulation may cause an advance or delay in endometrial maturation in the early or mid-luteal phase [24-27]. Studies investigating the relationship between sonographic measurements of the endometrium in the luteal phase and histologic dating of the endometrial biopsy have found no correlation [6, 7, 22, 28, 29]. Some studies have found the absence of the HH pattern in the luteal phase to be an indication for further evaluation [6, 7]. However, due to the risk of possibly disturbing a pregnancy once ET has been performed, we cannot either corroborate or dispute these findings. One possible future treatment plan could include an endometrial biopsy in the next cycle, using the same endometrial preparation protocol without performing ET. Another option could be to perform an endometrial biopsy on all women prior to their frozen ET. Both of these options would rely on the premise that all conditions will remain in all replacement cycles.

We included the measurement of uterine blood flow resistance to determine if there was any replacement between luteal phase echo patterns and blood flow. A study by Sterzik *et al.* found no relationship between mid-luteal E2 and P levels, endometrial thickness, or RI and histologic dating of the mid-luteal endometrial biopsy but did find a significantly higher PI when the biopsy was in phase [22]. In the present study, no relationship could be established between blood flow and echo pattern or outcome. Prevalence of a non-HH pattern was 32.9% if the PI was > 3.5 and 30.4% of the RI was > .95. This was slightly higher than the incidence of an HH pattern for all the women (25.8%) but not statistically significant. However, the clinical PRs were also slightly higher at 40.4% (PI > 3.5) and 45.1% (RI > .95) compared to the rate of 38.8% for all women (p = NS).

One argument on the clinical value of this study is that it is based on occurrences after ET has been performed. Conversely, other studies evaluate parameters prior to ET when the decision to defer ET can be made. Many women in our center have enough cryopreserved embryos for more than one ET; therefore, if a non-HH echo pattern is seen and a pregnancy is not established on the first frozen ET cycle, modifications can be made in succeeding cycles. One other alternative could be to increase the P dosage in the cycle of observation to see if PRs would

increase; however, a randomized study would be needed to investigate this theory.

The PR was still acceptable at 38.8% when a HH pattern was not observed three days after ET. No factor could be found that can identify which patients with a trilaminar echo pattern after ET will not become pregnant.

Conclusions

Significantly lower clinical and ongoing PRs and implantation rates, in a larger series of patients than previously reported, are associated with a non-homogeneous hyperechogenic endometrial echo pattern seen sonographically three days after transfer in frozen ET cycles. Further study is needed to determine the cause of this poor prognosticating factor so that a method of treatment may be found to correct it.

References

- [1] Fleischer A.C., Kalemieris G.C., Entman S.S.: "Sonographic depiction of the endometrium during normal cycles". *Ultrasound Med. Biol.*, 1986, 12, 271.
- [2] Yoshimitsu K., Nakamura G., Nakano H.: "Dating sonographic endometrial images in the normal ovulatory cycle". *Int. J. Gynaecol. Obstet.*, 1989, 28, 33.
- [3] Lenz S., Lindenberg S.: "Ultrasonic evaluation of endometrial growth in women with normal cycles during spontaneous and stimulated cycles". *Hum. Reprod.*, 1990, 5, 377.
- [4] Takeuchi H., Itoh S., Fukuda M., Yoshida K., Ishi K., Takeuchi H., et al.: "Comparison of transvaginal sonographic appearance and endometrial histology". *Nippon Sanka Fujinka Gakkai Zasshi*, 1991, 43, 266.
- [5] Bakos O., Lundkvist O., Bergh T.: "Transvaginal sonographic evaluation of endometrial growth and texture in spontaneous ovulatory cycles: a descriptive study". *Hum. Reprod.*, 1993, 8, 799.
- [6] Grunfeld L., Walker B., Bergh P.A., Sandler B., Hofmann G., Navot D.: "High-resolution endovaginal ultrasonography of the endometrium: a noninvasive test for endometrial adequacy". *Obstet. Gynecol.*, 1991, 78, 200.
- [7] Doherty C.M., Silver B., Binor Z., Moto M.W., Radwanska E.: "Transvaginal ultrasonography and the assessment of luteal phase endometrium". *Am. J. Obstet. Gynecol.*, 1993, 168, 1702.
- [8] Check J.H., Dietterich C., Lurie D.: "Non-homogeneous hyperechogenic pattern 3 days after embryo transfer is associated with lower pregnancy rates". *Hum. Reprod.*, 2000, 15, 1069.
- [9] Check J.H., Gandica R., Dietterich C., Lurie D.: "Evaluation of a nonhomogeneous endometrial echo pattern in the midluteal phase as a potential factor associated with unexplained infertility". *Fertil. Steril.*, 2003, 79, 590.
- [10] Baker A.F., Check J.H., Hourani C.L.: "Survival and pregnancy rates of pronuclear stage human embryos cryopreserved and thawed using a single step addition and removal of cryoprotectants". *Hum. Reprod. Update*, 1997, 2 (CD-ROM).
- [11] Turnbull L.W., Lesny P., Killick S.R.: "Assessment of uterine receptivity prior to embryo transfer: a review of currently available imaging modalities". *Hum. Reprod. Update*, 1995, 1, 505.
- [12] Zaidi J., Campbell S., Pittrof R., Tan S.L.: "Endometrial thickness, morphology, vascular penetration and velocimetry in predicting implantation in an in vitro fertilization program". *Ultrasound Obstet. Gynecol.*, 1995, 6, 191.
- [13] Bohrer M.K., Hock D.L., Rhoads G.G., Kemmann E.: "Sonographic assessment of endometrial pattern and thickness in patients treated with human menopausal gonadotropins". *Fertil. Steril.*, 1996, 66, 244.
- [14] Friedler S., Schenker J.G., Herman A., Lewin A.: "The role of ultrasonography in the evaluation of endometrial receptivity following assisted reproductive treatments: a critical review". *Hum. Reprod. Update*, 1996, 2, 323.
- [15] Oliveira J.B., Baruffi R.L., Mauri A.L., Petersen C.G., Borges M.C., Franco J.G. Jr.: "Endometrial ultrasonography as a predictor of pregnancy in an in-vitro fertilization programme after ovarian stimulation and gonadotrophin-releasing hormone and gonadotrophins". *Hum. Reprod.*, 1997, 12, 2515.
- [16] Dietterich C., Check J.H., Choe J.K., Nazari A., Lurie D.: "Increased endometrial thickness on the day of human chorionic gonadotropin injection does not adversely affect pregnancy or implantation rates following in vitro fertilization-embryo transfer". *Fertil. Steril.*, 2002, 77, 781.
- [17] Kovacs P., Matyas S., Boda K., Kaali S.G.: "The effect of endometrial thickness on IVF/ICSI outcome". *Hum. Reprod.*, 2003, 18, 2337.
- [18] Laasch C., Puscheck E.: "Cumulative embryo score, not endometrial thickness, is best for pregnancy prediction in IVF". *J. Assist. Reprod. Genet.*, 2004, 21, 47.
- [19] Check J.H., Dietterich C., Graziano V., Lurie D., Choe J.K.: "Effect of maximal endometrial thickness on outcome after frozen embryo transfer". *Fertil. Steril.*, 2004, 81, 1399.
- [20] Check J.H., Lurie D., Dietterich C., Callan C., Baker A.: "Adverse effect of a homogeneous hyperechogenic endometrial sonographic pattern, despite adequate endometrial thickness on pregnancy rates following in-vitro fertilization". *Hum. Reprod.*, 1993, 8, 1293.
- [21] Johannisson E., Parker R.A., Landgren B.M., Diczfalusy E.: "Morphometric analysis of the human endometrium in relation to peripheral hormone levels". *Fertil. Steril.*, 1982, 38, 564.
- [22] Sterzik K., Abt M., Grab D., Schneider V., Strehler E.: "Predicting the histologic dating of an endometrial biopsy specimen with the use of Doppler ultrasonography and hormone measurements in patients undergoing spontaneous ovulatory cycles". *Fertil. Steril.*, 2000, 73, 94.
- [23] Ohno Y., Fujimoto Y.: "Endometrial oestrogen and progesterone receptors and their relationship to sonographic appearance of the endometrium". *Hum. Reprod. Update*, 1998, 4, 560.
- [24] Garcia J.E., Acosta A.A., Hsiu J.-G., Jones H.S. Jr.: "Advanced endometrial maturation after ovulation induction with human menopausal gonadotropin/human chorionic gonadotropin for in vitro fertilization". *Fertil. Steril.*, 1984, 41, 31.
- [25] Forman R.G., Eychenne B., Nessman C., Frydman R., Robel P.: "Assessing the early luteal phase in in vitro fertilization cycles: relationships between plasma steroids, endometrial receptors, and endometrial histology". *Fertil. Steril.*, 1989, 51, 310.
- [26] Basir G.H., O W.S., Ng F.H.Y., Ho P.C.: "Morphometric analysis of peri-implantation endometrium in patients having excessively high oestradiol concentrations after ovarian stimulation". *Hum. Reprod.*, 2001, 16, 435.
- [27] Saadat P., Boostanfar R., Slater C.C., Tourgeman D.E., Stanczyk F.Z., Paulson R.J.: "Accelerated endometrial maturation in the luteal phase of cycles utilizing controlled ovarian hyperstimulation: impact of gonadotropin-releasing hormone agonists versus antagonists". *Fertil. Steril.*, 2004, 82, 167.
- [28] Ficicioglu C., Tasdemir S., Arioglu P.F., Unlu R., Yorganci C.: "The use of transvaginal ultrasonography in the evaluation of luteal phase endometrium". *Acta Eur. Fertil.*, 1995, 26, 35.
- [29] Sterzik K., Grab G., Schneider V., Strehler E.J., Gagsteiger F., Rosenbusch B.E.: "Lack of correlation between ultrasonography and histologic staging of the endometrium in in vitro fertilization (IVF) patients". *Ultrasound Med. Biol.*, 1997, 23, 165.

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