A comparison of early gestational age markers in viable twin pregnancies resulting from in vitro fertilization-embryo transfer versus spontaneous conception with ovarian stimulation

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Summary

The objective of this study was to determine whether there were differences in gestational age markers in dizygotic twin pregnancies resulting from in vitro fertilization-embryo transfer (IVF-ET) when compared with dizygotic twin pregnancies spontaneously conceived following ovulation inducing therapy drugs without assisted reproductive techniques (ART). Thirty-one sets of twins conceived by IVF-ET and 33 sets of twins conceived without ART were monitored by serum beta human chorionic gonadotropin (B-hCG) levels and transvaginal sonographic measurements of sac size (SS) and crown-rump length (CRL). Comparison between groups found that SS was significantly smaller in the IVF-ET group as compared to the non-ART group 21-35 days post-ovulation ($p<.05$). The CRL was similar in both groups as well as the doubling times of B-hCG. These data indicate that in dizygotic twin pregnancies initial development of SS may be slower following IVF-ET than in spontaneously conceived twins.

Key words: Twins; IVF-ET; Sonography; Gestational sac; Crown-rump length; B-hCG; Implantation.

Introduction

Some reports have suggested that implantation of embryos from in vitro fertilization-embryo transfer (IVF-ET) may be delayed when compared to implantation occurring from in vivo fertilized eggs [1-2]. Other researchers have sought either to dispute or substantiate this thereby comparing methods of evaluating early pregnancy [3]. One such tool is the serum B-hCG level which can detect implantation as early as 8-10 days after the luteinizing hormone (LH) peak [1-3]. Reports from some centers have indicated that evidence of implantation is not identified until 12-13 days after the administration of human chorionic gonadotropin (hCG) in IVF conceptions [2, 3]. Beta (B)-hCG levels may be used not only to diagnose implantation but also to follow the progression of a pregnancy by repeating the assay and following doubling times.

Perhaps one of the most beneficial tools in evaluating and monitoring pregnancy is sonography. High frequency vaginal transducers have made it possible to identify a pregnancy at much lower B-hCG levels than before [5]. In our experience, an intrauterine gestational sac can usually be identified 21-22 days post-ovulation and an embryo with evidence of cardiac motion is usually seen by 28-30 days post-ovulation. Two reports have failed to demonstrate evidence of delayed implantation in IVF-ET pregnancies through sonographic documentation of early gestational sac size (SS) [3] and crown-rump length (CRL) [6]. These studies did not detect any differences between IVF-ET pregnancies and those achieved through non-assisted reproductive techniques (ART) in singleton pregnancies. The objective of our study was to compare these early gestational age markers in dizygotic twin pregnancies occurring following IVF-ET to those occurring without ART.

Materials and Methods

We studied 64 pregnancies with 2 viable gestations; 33 were conceived following treatment with ovulation inducing drugs (clomiphene citrate or human menopausal gonadotropins) without ART, and 31 were conceived following IVF-ET. In all cases two gestational sacs were initially observed sonographically and two viable embryos were seen in follow-up. Gestational age was computed from day of ovulation. In patients treated without ART, day of ovulation was determined by serial ultrasound folliculograms and serum estadiol (E_2) and progesterone (P) levels [7, 8]. Ovulation was presumed to occur 24 hours after the peak serum E_2 was attained or the administration of hCG. Ovulation was confirmed by demonstration of the collapse of at least one mature follicle (18-24 millimeters in diameter) by at least 5 mm [9]. In IVF-ET cycles, day of oocyte retrieval was used as the day of ovulation.

Transvaginal fetal sonography was performed on an ATL Ultramark 4 equipped with a 5 MHz endovaginal transducer (Advanced Technology Laboratories, Bothell, WA) at regular intervals 28-42 days post-conception. Measurement of early gestational development included measurements of the gestational SS and CRL. Mean SS was calculated in millimeters from an average of the length, width and height of the gestational sac, as measured from the inner wall [10]. Crown-rump length in millimeters was measured as the greatest length of the embryo [11]. The gestational age and actual and expected SS

Table 1. — *Comparison of discrepancy scores for sac size by conception methods*

# days post-ovulation		IVF-ET twins	Non-ART twins
21-28 days	Twin 1	−1.2±2.5 mm*	.8±2.3 mm*
	Twin 2	−1.6±1.9 mm*	1.0±2.2 mm*
29-35	Twin 1	−2.0±3.9 mm*	1.9±3.1 mm*
	Twin 2	−2.1±3.4 mm*	2.0±2.1 mm*
36-42	Twin 1	−1.6±4.4 mm	−1.3±3.6 mm
	Twin 2	−5.3±5.2 mm	−2.5±5.2 mm

* $p < .05$, between group comparison

Table 2. — *Comparison of discrepancy scores for crown-rump length by conception methods**

# days post-ovulation		IVF-ET twins	Non-ART twins
21-28	Twin 1	−1.0±1.4 mm	−1.2±1.7 mm
	Twin 2	−0.4±1.1 mm	−1.5±1.3 mm
29-35	Twin 1	−1.1±2.3 mm	−0.5±1.0 mm
	Twin 2	−0.7±2.0mm	−0.1±1.2 mm
36-42	Twin 1	−2.6±1.4 mm	−1.3±3.6 mm
	Twin 2	−2.7±1.7 mm	−1.5±2.6 mm

p = NS

and CRL were recorded. Since all sonograms were not performed on the same post-ovulatory day, a comparison of discrepancy scores was considered more meaningful. Discrepancy scores were computed as the difference between the actual measurements and the expected norm (derived from singleton pregnancies in our practice) for that gestational age. Sac size and CRL discrepancy scores were compared at three time intervals: 21-28 days (T1), 29-35 days (T2), and 36-42 days (T3) post-ovulation [7].

Serum measurements of B-hCG were taken at regular intervals in the first four weeks of pregnancy. The first measurements was taken between 13 and 15 days post-ovulation and the second taken 2-4 days later. The doubling time was calculated from two serial B-hCG levels using the formula DT = (t2-t1)* ln(2)/ln(hCG2/hCG1), where t1 = day of first serum level; t2 = day of second serum level, hCG1 = hCG level measured at t1; hCG2 = hCG level measured at t2, ln denotes the natural logarithm [4].

Statistical analysis of the data included independent t-test to compare mean outcome variables between IVF-ET and non-ART twins; paired t-test to compare means within sets of twins. A p value of $< .05$ was used to determine significance.

Results

The mean age of the women who conceived twins without ART was 31.4±3.5 years; those conceiving through IVF-ET were older, with a mean age of 34-55 years.

Sac Size:

During T1 (21-28 days post-ovulation) and T2 (29-35 days post-ovulation), the SS was larger than expected in the non-ART group and smaller than expected for the IVF-ET group ($p<.05$). By T3 (36-42 days post-ovulation) these differences were no longer observed (Table 1). Within each group there was no difference between the SS of the twins (p = NS).

Crown-rump Length:

The CRL measurements were all smaller than expected. There was no difference between the discrepancy scores when comparing the two groups. There was no difference between the discrepancy scores of the two fetuses in each pregnancy in both non-ART and IVF-ET pregnancies (Table 2).

Beta-Human Chorionic Gonadotropin Doubling Time:

The B-hCG doubling time was 1.8±.7 days for the non-ART twins and 1.7±.6 days for the IVF-ET twins (p = NS).

Discussion

The only parameter to differ between non-ART and IVF-ET dizygotic twins in early gestation was the sac size prior to 36 days after ovulation. These data may be indicative of a delayed implantation mechanism associated with IVF, at least with twins, contrary to the findings of Pellicer *et al.*, and Rossavik *et al.*, in singleton pregnancies [3, 6]. One difficulty in comparing IVF-ET with non-ART pregnancies is in determining date of conception. In IVF-ET pregnancies varying days have been considered to represent day 0; some consider it to be the day of hCG administration [3], while others have based their studies on day of oocyte retrieval (12] (as in the present study) or the day of ET [13]. The first two methods can be more easily applied because these days are constant with the exception of possibly a few hours variation of the time from hCG administration to oocyte retrieval [14]. Day of ET can have a one day variaiton depending on the age of the embryos at the time of ET.

When determing the day of ovulation in non-ART cycles with multiple follicles, ovulation can occur over a span of 110 hours from the LH surge [15]. We have found 24 hours from peak E_2 and follicular maturity to provide the best correlation. This may be an underestimation of time of ovulation but does not compromise the validity of our study, since if this is indeed an underestimation, the non-ART twins would have measured smaller than expected for dates.

No differences in CRL discrepancy scores were noted between IVF-ET and non-ART twin gestations. These data are consistent with the findings of Rossavik *et al.*, but cannot be compared with the data published by Pellicer *et al.*, since they did not compare the CRL. Their embryonic comparison related to the timing of the presence of a heartbeat.

No differences in B-hCG doubling times were found between the groups and these doubling times were not different from those of singleton pregnancies. Doubling times have not been found to be predictive of determining normal twin versus singleton pregnancies [4] nor do they correlate with sac size.

Conclusions

The mechanism for small for dates sac sizes for IVF twins is not known. One possibility is delayed implantation. Whatever the mechanism, the important message of the study is that the clinician should not interpret small

for dates sac sizes in dizygotic IVF twins as evidence of spontaneous abortion and decide to stop P support, or even worse, set up a D and E.

References

[1] Lenton E. A., Neal L. M., Sulaiman R.: "Plasma concentrations of human chorionic gonadotropin from the time of implantation until the second week of pregnancy". *Fertil. Steril.*, 1982, *37*, 773.

[2] Englert Y., Roger M., Belaisch-Allart J., Jondet M., Frydman R., Testart J.: "Delayed appearance of plasmatic chorionic gonadotropin in pregnancies after in vitro fertilization and embryo transfer". *Ferti. Steril.*, 1984, *42*, 835.

[3] Pellicer A., Calatayad C., Miro F., Castellvi R. M., Ruiz A., Ruiz M., Perez M., Bonilla-Musoles F.: "Comparison of implantation and early development of human embryos fertilized in vitro versus in vivo using transvaginal ultrasound". *J. Ultrasound Med.*, 1991, *10*, 31.

[4] Check J. H., Weiss R. M., Lurie D.: "Analysis of serum human chorionic gonadotrophin levels in normal singleton, multiple and abnormal pregnancies". *Hum. Reprod.*, 1992, *7*, 1176.

[5] Bernaschek G., Rudelstorfer R., Csaicsica P.: "Vaginal sonography versus serum chorionic gonadotropins in early detection of pregnancy". *Am. J. Obstet. Gynecol.*, 1988, *158*, 608.

[6] Rossavik I. K., Torjusen G. D., Gibbons W. E.: "Conceptual age and ultrasound measurements of gestational sac and crown-rump length in in-vitro fertilization pregnancies". *Fertil. Steril.*, 1988, *49*, 1012.

[7] Nazari A., Check J. H., Epstein R. H., Dietterich C., Farzanfar S.: "Relationship of small-for-dates sac size to crown-rump length and spontaneous abortion in patients with a known date of conception". *Obstet. Gynecol.*, 1991, *78*, 369.

[8] Check J. H., Nowroozi K., Wu C. H., Adelson H. C., Lauer C.: "Ovulation-inducing drugs versus progesterone therapy for infertility in patients with luteal phase defects". *Int. J. Fertil.*, 1988, *33*, 252.

[9] Check J. H., Dietterich C., Nowroozi K., Wu C. H.: "Comparison of various therapies for the luteinized unruptured follicle (LUF) syndrome". *Int. J. Fertil.*, 1992, *37*, 33.

[10] Hellman L. M., Kobayashi M., Fillisti L., Lavenbar M.: "Growth and development of the human fetus prior to the twentieth week of gestation". *Am. J. Obstet. Gynecol.*, 1969, *103*, 787.

[11] Robinson H. P., Fleming J. E. E.: "A critical evaluation of sonar crown-rump length measurements". *Br. J. Obstet. Gynaecol.*, 1975, *82*, 701.

[12] Dickey R. P., Gasser R. F.: "Ultrasound evidence for variability in the size and development of normal human embryos before the tenth post-insemination week after assisted reproductive technologies. *Hum. Reprod.*, 1993, *8*, 331.

[13] Wax M. R., Frates M., Benson C., Yeh J., Doubilet P. M.: "First trimester findings in pregnancies after in vitro fertilization". *J. Ultrasound Med.*, 1992, *11*, 321.

[14] Summers D., Check J. H., Hoover L., O'Shaughnessy A., Nazari A.: "A 36 hour time of retrieval from human chorionic gonadotropin (hCG) injection may produce better pregnancy rates (PR) than a 34 hour retrieval". IX World Congress on in vitro fertilization and assisted reproduction, Monduzzi Editore, Edited by A. Aburumieh, E. Bernat, G. Dohr, W. Feichtinger, F. Fischl, J. Huber, E. Muller, S. Szalay, W. Urdl, H. Zech, 1995, 291.

[15] Navot D., Margalioth E. J., Laufer N., Mor-Yosef S., Schenker J. G.: "Asynchronous ovulation in human menopausal gonadotropin induction of ovulation for in vitro fertilization". *Fertil. Steril.*, 1984, *42*, 806.

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