FERTILITY AND STERILITY®
VOL. 73, NO. 6, JUNE 2000

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer

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Objective: To determine the relationship between blastocyst score and pregnancy outcome.

Design: Retrospective review of blastocyst transfer in an IVF clinic.

Setting: Private assisted reproductive technology unit.

Patient(s): 107 patients undergoing blastocyst culture and transfer of two embryos.

Intervention(s): Culture of all pronucleate embryos in sequential media to the blastocyst stage (day 5), followed by transfer of two blastocysts.

Main Outcome Measure(s): Implantation rates, pregnancy rates, and twinning were analyzed.

Result(s): When a patient received two top-scoring blastocysts (64% of patients), implantation and pregnancy rates were 70% and 87%, respectively. The twinning rate in this group was 61%. When only one top-quality blastocyst was available for transfer (21% of patients), the implantation and pregnancy rates were 50% and 70%. The twinning rate for this group was 50%. In contrast, when only low-scoring blastocysts were available for transfer (15% of patients), implantation and pregnancy rates were 28% and 44%, and the twinning rate was 29%. No monozygotic twins were observed in this group of patients.

Conclusion(s): The ability to transfer one high-scoring blastocyst should lead to pregnancy rates greater than 60%, without the complication of twins. (Fertil Steril® 2000;73:1155–8. ©2000 by American Society for Reproductive Medicine.)

Key Words: ART, embryo culture, implantation, IVF, viability

The advent of routine blastocyst culture and transfer using sequential culture media in clinical IVF has been associated with a significant increase in implantation rates, both in patients with a good response to gonadotropins (1, 2) and in nonselected patients (3). In the former patients, blastocyst culture and transfer has been an effective means of eliminating high-order multiple gestations (4). However, concomitant with increases in implantation rates, around half of the resulting pregnancies are twins even when only two blastocysts are transferred (1). The only effective way of eliminating twins is to transfer a single embryo; it is therefore necessary to develop an effective way of identifying the most viable embryo in a given cohort (5).

Gardner and Schoolcraft (6) developed a three-part scoring system based on blastocyst expansion, inner cell mass, and trophectoderm development in an attempt to grade human blastocysts before transfer. We report here a retrospective analysis of patients receiving blastocysts on day 5 and the correlation of implantation and pregnancy outcome with respect to the embryo score.

MATERIALS AND METHODS

One hundred seven patients having blastocyst culture and transfer between June 1998 and August 1999 were included in the analysis. Requirements for inclusion in this analysis were [1] basal follicle-stimulating hormone level <15 mIU/mL, [2] age <45 years, [3] presence of normal uterine cavity, [4] adequate semen variables for IVF or ICSI, [5] at least 10 follicles ≥ 12 mm in diameter visible by trans-

Received October 13, 1999; accepted December 21, 1999.

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0015-0282/00/$20.00
PII S0015-0282(00)00518-5

vaginal ultrasonography on the day of hCG administration, and [6] two blastocysts of any grade available for transfer. No oocyte donor patients were included in this analysis. Institutional review board approval was not obtained, as this was a retrospective analysis of case results.

Down-regulation was accomplished by using leuprolide acetate (Lupron; TAP Pharmaceuticals, North Chicago, IL), 1.0 mg/d, s.c. from the midluteal phase for 10 days; the dosage was then reduced to 0.5 mg/d until the morning of hCG injection. Administration of hMG was begun after pituitary down-regulation (E_2 level $<$35 pg/mL [128 pmol/L]) and was continued until at least 10 follicles reached a mean diameter of 12 mm. Oocyte retrieval was scheduled for 35 hours after hCG injection.

Embryo Culture

Media G 1.2 and G 2.2 containing 5 mg/mL of human serum albumin (1) were prepared weekly in the laboratory and were screened before use with a mouse embryo bioassay (7). Semen preparation was carried out by using a 50-70-95 discontinuous gradient of Pure Sperm (Nidacon, Gothenburg, Sweden). The resulting pellet was washed in fertilization medium (8) and stored in the incubator until insemination. One hundred thousand sperm per milliliter were added to each oocyte. If ICSI was performed, all oocytes were denuded by using hyaluronidase and drawn pipettes. Each mature oocyte was placed in a 6-μL droplet of phosphate-buffered saline supplemented with 15% fetal cord serum.

The partner's sperm was placed in a 6-μL droplet of PVP (IVF Science Scandinavia, Gothenburg, Sweden). All droplets were overlaid with paraffin oil (BDH, Poole, Dorset, UK). Intracytoplasmic sperm injection was performed on a Nikon inverted microscope (Nikon, Melville, NY) with Narashige micromanipulators (Narashige, East Meadow, NY). Injected oocytes were then rinsed and placed in tubes of G 1.2 until fertilization was assessed. Assessment of fertilization took place 15–18 hours after insemination. Cumulus and corona cells were removed by dissection with 27-gauge disposable needles in an organ culture dish. All gamete and embryo manipulations occurred in a pediatric isolette designed to control humidity, temperature, and pH fluctuations.

Embryos with two pronuclei were cultured in groups of three or four in 1 mL of medium G 1.2 in prerinsed 5-mL Falcon tubes. Around noon on day 3, all embryos were transferred to 1 mL of medium G 2.2 for a further 48 hours of culture (day 5 of development). On the morning of day 5, the percentage of blastocyst formation was determined and each blastocyst was assigned a score by using the system of Gardner and Schoolcraft (6). Briefly, blastocysts were given a numerical score from 1 to 6 on the basis of their degree of expansion and hatching status, as follows: 1, an early blastocyst with a blastocoel that is less than half of the volume of the embryo; 2, a blastocyst with a blastocoel that is half of or greater than half of the volume of the embryo; 3, a full blastocyst with a blastocoel completely filling the embryo; 4, an expanded blastocyst with a blastocoel volume larger than that of the early embryo, with a thinning zona; 5, a hatching blastocyst with the trophectoderm starting to herniate though the zona; and 6, a hatched blastocyst, in which the blastocyst has completely escaped from the zona.

For blastocysts graded as 3–6 (i.e., full blastocysts onward), the development of the inner cell mass was assessed as follows: A, tightly packed, many cells; B, loosely grouped, several cells; or C, very few cells. The trophectoderm was assessed as follows: A, many cells forming a cohesive epithelium; B, few cells forming a loose epithelium; or C, very few large cells. By using this scoring system, two blastocysts were selected for transfer. Blastocysts were transferred in medium G 2.2 early in the afternoon; those that were not transferred were cryopreserved. No embryos in the blastocyst group underwent assisted hatching or complete zona removal.

Embryo Transfer and Luteal-Phase Support

Transfers were performed by using a Wallace catheter (Edwards-Wallace catheter; Marlow Technologies, Inc., Willoughby, OH) and ultrasonographic guidance. Methylprednisolone (16 mg once per day) and tetracycline (250 mg four times per day) were administered to all patients for 4 days beginning on the day of oocyte retrieval. Luteal support involved intramuscular administration of 50 mg of progesterone in oil initiated 2 days after oocyte retrieval.

Statistical Analysis

Mean age of patients was examined by using analysis of variance followed by the Bonferonni procedure for multiple comparisons. Percentage data was analyzed by using the Fischer exact test or χ^2 test for trend, as appropriate.

RESULTS

Patients were assigned to one of three groups according to the quality of their blastocysts transferred on day 5. Patients in group 1 had two top-scoring blastocysts available for transfer ($\geq$3AA). Patients in group 2 had one top-scoring blastocyst ($\geq$3AA), and patients in groups 3 did not have a top-scoring blastocyst for transfer. Blastocyst score had a significant effect on implantation and pregnancy rates and on twinning (Table 1). There were no significant differences between the causes of infertility in the three groups (Table 2).

During the period of this analysis, 34 patients had their blastocysts scored and transferred but for personal reasons had either one or three blastocysts replaced. Five patients (3.5% of the patients during the study period) had only one blastocyst transferred. Three patients who conceived after the transfer of a single embryo had blastocysts with scores of 3AA, 4AA, or 5AA transferred. In contrast, the two patients

TABLE 1

Effect of blastocyst score on pregnancy outcome.

Variable	Patient group		
	Group 1 (two blastocysts ≥3AA)	Group 2 (one blastocyst ≥3AA)	Group 3 (blastocysts <3AA)
No. of embryos transferred	2	2	2
Mean age (±SE) (y)	32.9 ± 0.5	33.2 ± 1.0	33.3 ± 0.9
Age range (y)	25–43	21–41	30–39
No. of transfers	68	23	16
Patients with ICSI (%)	37	43	38
No. of 2PN embryos	1,029	288	201
No. of blastocysts	587	134	67
Blastocyst development from 2PN (%)	57.0[a]	46.5[b]	33.3
No. of fetal sacs	99	25	9
Implantation rate (% sacs)[cd]	72.8	54.3	28.1
No. of fetal hearts	95	23	9
Implantation rate (% with fetal heart beat)[cd]	69.9	50.0	28.1
No. of pregnant patients	59	16	7
Clinical pregnancy rate (%)[c]	86.8	69.6	43.8
No. of twins	36	8	2
Twin (%)	61.0	50.0	28.6

Note: See text for explanation of blastocyst scoring system.

[a] $P<.001$ (group 1 vs. group 2).

[b] $P<.01$ (group 2 vs. group 3).

[c] $P<.001$ (significant linear trend among all groups).

[d] Implantation rates per blastocyst transferred were calculated from all patients who received embryos, not just those who subsequently conceived.

Gardner. Blastocyst score and pregnancy. Fertil Steril 2000.

who did not conceive did not have a top-scoring blastocyst: One received a grade 2, and the other received a 3BB. Twenty-nine patients had three blastocysts replaced. When three low-scoring blastocysts were transferred (19 patients), no difference was seen in the clinical pregnancy rate (36.8%) compared with the rate among patients in whom two low-scoring blastocysts were transferred (44%).

No monozygotic twins were detected in the patient population by ultrasonography.

DISCUSSION

It is evident from this analysis that the quality of the human blastocyst, as quantitated by a systematic scoring system, can be used to identify viable embryos for transfer. When a patient had two good-quality blastocysts available for transfer (group 1, representing 64% of patients), implantation rates (as determined by the presence of a fetal heart) of 70% were attained, with a 61% incidence of twins. Implantation rates were calculated for all patients who had an

TABLE 2

Causes of infertility.

Cause of infertility	No. (%) of patients with indicated cause of infertility		
	Group 1 (two blastocysts ≥3AA)	Group 2 (one blastocyst ≥3AA)	Group 3 (blastocysts <3AA)
Male factor	17 (25.0)	3 (13.0)	4 (25)
Tubal factor	10 (14.7)	3 (13.0)	2 (12.5)
Endometriosis	18 (26.5)	7 (30.5)	3 (18.75)
Ovarian disorders	15 (22.0)	6 (26.2)	3 (18.75)
Idiopathic/unexplained	4 (5.9)	1 (4.3)	1 (6.25)
Other	4 (59)	3 (13.0)	3 (18.75)

Note: There were no statistically significant differences in the causes of infertility between the patients in the different groups. See text for explanation of blastocyst scoring system.

Gardner. Blastocyst score and pregnancy. Fertil Steril 2000.

embryo transfer, not just those who subsequently became pregnant. It is therefore recommended that in patients with a top-scoring blastocyst, transfer of a single blastocyst should be considered.

Discussion about whether blastocyst culture is advantageous merely because of increased embryo selection continues. Several groups have proposed that instead of blastocyst culture and transfer, the quality of embryos should be assessed at the pronucleate stage (9) or by using more rigorous scoring systems on day 3 (10, 11). With such approaches, implantation rates of 28% and 48% have been reported for pronucleate and day 3 embryos, respectively. Such figures compare favorably to those obtained with blastocyst transfer 55% (1), questioning the necessity for extended culture. However, in the study of Gerris et al. (11), a highly selected group of patients was recruited—women younger than 34 years of age and who had had no previous IVF cycles. Furthermore, in the studies of Scott and Smith (9) and of Gerris et al., (11), fewer than half of the patients in the analysis were used to calculate the high implantation rates. In the present study, it has been shown that for this patient population, much higher implantation rates can be achieved, and that by scoring blastocysts one can identify those with the highest probability of implantation. Around 50% of patients entering our program fulfill the criteria set for this analysis.

The implantation rate of 70% with use of two top-scoring blastocysts is substantially higher than that achieved in the selected groups used to establish implantation rates for pronucleate (28%) and cleavage stage embryos (48%). This finding should therefore lead to the introduction of single-blastocyst transfers for patients with at least one quality blastocyst, as pregnancy rates greater than 60% should be established without the complication of twins. In support of this, patients who received a single top-scoring blastocyst in our analysis all conceived. Furthermore, in patients who do not have a top-quality blastocyst, transfer of two blastocysts may be warranted on the basis of the observed lower implantation rate of such embryos. This method is similar to that of Steer et al. (12), who produced a cumulative embryo scoring system for cleavage-stage embryos. In patients who elected to have three embryos transferred, there appeared to be no benefit in the transfer of a third blastocyst, which by default exposes the patients to the possible risk of conceiving triplets. The ability to assign more exact implantation rates to specific blastocyst scores will greatly assist the model proposed by Martin and Welch (13) in maximizing pregnancy rates while being able to predict the probability of a multiple gestation should more than one blastocyst be transferred.

In conclusion, blastocyst culture and transfer have previously been shown to be an effective means of eliminating high-order multiple gestations and thereby avoiding the many problems associated with such pregnancies (1, 4). This analysis has revealed that the quality of individual human blastocysts can be quantitated by using a three-part scoring system, which can then be used to select blastocysts for transfer. On the basis of the implantation potential of top-quality blastocysts, it is suggested that a substantial number of patients would benefit from single-blastocyst transfers. To test this hypothesis, a prospective, randomized trial is currently under way at the Colorado Center for Reproductive Medicine.

Acknowledgments: The authors thank Mike Baird, Marsha Gee, Susie Guadagnoli, Mary Rawlins, Lyla Wagley, and all staff members at the Colorado Center for Reproductive Medicine for their support.

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