

Pregnancy rates (PRs) according to embryo cell number at time of embryo transfer (ET)

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Summary

Purpose: To evaluate pregnancy and implantation rates following fresh and frozen embryo transfer (ET) according to blastomere number.

Methods: A retrospective study from 1/1/97 to 9/30/98 including all cycles with ETs irrespective of age.

Results: 65% of fresh transfers had at least one 8-cell embryo vs only 39.6% for frozen ET. The clinical pregnancy and implantation rates were higher when one 8-cell embryo was transferred (64% and 24%) vs a 5-7 cell embryo (41% and 14.5%) for fresh transfers. There was less of a difference with frozen ETs (46% and 19% for 8-cell vs 38% and 17% for 5-7 cell).

Conclusions: Since mostly only 8-cell embryos at day 3 reach the blastocyst stage, these data raise questions as to whether the quest to attain the highest pregnancy rate per transfer through blastocyst transfer, may be at the expense of overall pregnancy rate (fresh and frozen) from a given oocyte harvest.

Key words: Blastocysts; Blastomere number; Cryopreservation.

Introduction

The advent of new embryo culture medias has allowed successful development of human blastocysts without the need of co-cultures. This has lead to a significant rise in the percentage of in vitro fertilization (IVF) centers transferring 5-6 day-old blastocysts. One obvious advantage is a high implantation rate per embryo which allows the transfer of fewer embryos and thus a reduction in the rate of multiple gestations.

What is not clear is whether one can assume that an embryo not making the blastocyst stage would not have resulted in a viable pregnancy had it been transferred at the 2nd or 3rd day-old stage. Though some IVF centers report that 93% of patients have at least one blastocyst to transfer, it is not unusual for some centers to report 40% of the patients not to even have an embryo transfer (ET) [1].

Previous studies failed to find a strong correlation with embryo morphology of multi-cell embryos and subsequent pregnancy rates (PRs) [2]. Similarly, the predictive value of embryo morphology on day 3 for subsequent blastocyst formation is limited [3]. A positive correlation was observed with the number of 8-cell embryos formed and subsequent blastocyst formation [1].

To help determine if culturing to blastocyst stage may help select the best embryos so that the PR and implantation on first transfer improves at the expense of a decrease in overall PRs per oocyte retrieval, we thought it would be useful to determine the PRs and implantation rates according to embryo cell number at the time of a 3-day transfer. Furthermore, comparisons would be

made between transfers on retrieval cycles vs frozen ET according to cell number of the embryos at the time of transfer.

Materials and Methods

A retrospective study of all ET cycles performed at our IVF center between 1/1/97 and 9/30/98 were included in this study. Six hundred and seventy-eight cycles followed oocyte retrieval (fresh transfers) and 522 cycles involved the transfer of cryopreserved embryos (frozen ETs).

There were no exclusions for age, follicular phase follicle stimulating hormone (FSH) levels, type of ovarian hyperstimulation protocol used, etiology of infertility, or number of previous ETs. Ovarian hyperstimulation protocols included both luteal phase and follicular phase administration of leuprolide acetate and ovarian stimulation with either all recombinant FSH, all human menopausal gonadotropin (hMG), or a mixture of the two.

Embryo selection was used when transferring either fresh or frozen embryos by allowing twice as many embryos as intended to transfer to cleave to the 72 hour stage, and the best half, based on number of blastomeres and fragmentation, were transferred; those remaining were either frozen or refrozen (or discarded if very poor quality). The extra embryos not in the group used for the selection of the embryos for fresh transfer were cryopreserved at the 2 pronuclear stage.

Embryos were cryopreserved and thawed using a simplified one-step procedure [4]. Assisted embryo hatching was performed on day-3 embryos as previously described [5, 6]. Frozen ET protocols included hormone replacement therapy with or without down regulation as needed. Typically it consisted of a gradual increase in oral estradiol (E2) dosage from 2-6 mg over a two week period. Sometimes the dosage started at 4 mg if a previous cycle showed poor endometrial development. Someti-

mes E2 patches were used when oral E2 was not tolerated. Progesterone (P) supplementation usually consisted of 200 mg, twice daily, P vaginal suppositories, and 100 mg IMP daily.

The cycles were grouped by the maximum cell stage in the embryo pool transferred. In group 1, all embryos were less than 4 cells. In group 2, the fastest growing embryo only reached a 4-cell stage. In group 3, the fastest growing embryo reached a 5 to 7 cell stage; in group 4, the fastest growing embryo reached an 8-cell stage, but only one embryo attained this stage; in group 5, at least two embryos were transferred at the 8-cell stage. This grouping was used because it was hypothesized that 8-cell embryos would be more likely to proceed to blastocyst and thus have a higher implantation rate. Results were also calculated individually for cycles where the maximum cell stage attained by an embryo was 4, 5, 6, 7 or 8 cells, respectively.

Main outcome measures were clinical PR per transfer and implantation rate. The PR and implantation rates for each cell stage group undergoing fresh ET were also compared by age and use of intracytoplasmic sperm injection (ICSI). There were not enough frozen ET cycles in older women and ICSI patients to perform statistical inference.

Chi-square analysis was used to compare the rates by cell stage groups. A p value of .05 was used. Significant associations were further analyzed by partitioning the chi-square into independent components (agresti). Due to the small sample size in group 1, this group was not included in the statistical inference.

No institutional review board approval was needed because this was a retrospective study. All patients initially signed a waiver that their cases can be used for compilation in research studies.

Results

Of the 678 fresh transfers performed, only 65.1% involved the transfer of at least one 8-cell embryo. Of the 522 frozen transfers, only 39.6% had at least one 8-cell embryo.

For fresh cycles, the clinical PRs were found to be associated with maximum cell stage of the embryos ($p < .05$, chi-square, Table 1). Although there was no difference in the rates if one or more 8-cell embryos were transferred, the rates were lower if the maximum cell stage was 5-7 ($p < .05$). They were also further reduced if the maximum cell stage was 4 ($p < .05$). The rate of multiple pregnancies was also highest (51.6%) in the groups with at least two 8-cell embryos transferred.

Implantation rates and cell stage were also associated ($p < .05$, chi-square). As seen in Table 1, the implantation rates were highest for cycles with at least one 8-cell embryo (23.7% and 24.9%). They were significantly reduced to 14.5% if the maximum cell stage attained was 5-7 cells ($p < .05$) and to 8.5% if the maximum cell stage was 4 cells ($p < .05$).

A similar association was found in frozen ETs (Table 2). The PRs were highest and similar if at least one 8-cell embryo was transferred (46.0% and 57.4%, grs 4 and 5, respectively). Rates were reduced to 38.1% if the maximum cell stage was 5-7 cells, and further reduced to 27.9% if the maximum cell stage was 4. The same trend was seen in implantation rates in the frozen ETs (Table 2).

Within each cell stage group, no significant differences were found between the PRs and implantation rates in fresh and frozen ETs. In both fresh and frozen cycles, there was a trend for the rate of multiple gestations to increase as the embryo development progressed. However, statistical significance was not attained.

Since there were no differences in the two groups in which 8-cell embryos were transferred, these groups were combined and PRs per transfer were compared by the maximum cell size in the embryo pool transferred. These results are presented in Table 3. Transfers with a maximum embryo cell stage of 7 performed similarly to those with 8-cell embryos, but PRs and implantation rates

Table 1. — Comparison of pregnancy rates by maximum cell stages of embryos transferred on cycle of retrieval (fresh transfer).

	Embryo Development				
	All embryos < 4 cells (gr 1)	One or more 4-cell embryos (gr 2)	One or more 5-7 cell embryos (gr 3)	One 8-cell embryo in batch (gr 4)	Two or more 8-cell embryos (gr 5)
# of transfers	3 (.4%)	36 (5.3%)	198 (29.2%)	212 (31.3%)	229 (33.8%)
Age (years)	39.0±6.1	36.2±4.3	36.6±5.7	36.5±5.3	36.3±5.2
# of embryos transferred ^a	2.0±0.0	2.3±1.3 ^b	3.3±1.3	3.4±1.1	3.7±.9
Pregnancies (total) ^a					
(%/transfer)	0 (0.0%)	5 (13.9%) ^c	81 (40.9%) ^b	136 (64.1%)	139 (60.7%)
Chemical	0 (0.0%)	0 (0.0%)	10 (5.1%)	6 (2.8%)	9 (3.9%)
Ectopic	0 (0.0%)	0 (0.0%)	4 (2.0%)	7 (3.3%)	6 (2.6%)
Clinical ^a	0 (0.0%)	5 (13.9%) ^c	67 (33.8%) ^b	113 (53.3%)	124 (54.1%)
Singleton (%/clinical)	0 (0.0%)	4 (80.0%)	43 (64.2%)	68 (60.2%)	60 (48.4%)
Multiple (%/clinical)	0 (0.0%)	1 (20.0%)	24 (35.8%)	45 (39.8%)	64 (51.6%)
Twins	0	0	20	30	38
Triplets	0	1	4	14	22
Quadruplets	0	0	0	1	4
Viable ^a	0 (0.0%)	4 (11.1%) ^c	62 (31.3%) ^b	102 (48.1%)	108 (47.2%)
Implantation rate ^a	0.0% (0/3)	8.5% (7/82) ^c	14.5% (95/656) ^b	23.7% (171/722)	24.9% (214/858)

^a $p < .05$, comparing groups 2 through 5; ^b $p < .05$, comparing groups 4 and 5 to group 3; ^c $p < .05$, comparing group 2 to groups 3, 4, and 5.

Table 2. — Comparison of pregnancy rates by maximum cell stages of embryos transferred - frozen embryo transfers.

	Embryo Development				
	All embryos < 4 cells (gr 1)	One or more 4-cell embryos (gr 2)	One or more 5-7 cell embryos (gr 3)	One 8-cell embryo in batch (gr 4)	Two or more 8-cell embryos (gr 5)
# of transfers	7 (1.3%)	43 (8.2%)	265 (50.8%)	139 (26.6%)	68 (13.0%)
Age (years)	38.1±5.4	36.5±6.8	36.8±6.3	36.6±5.7	36.7±6.9
# of embryos transferred ^a	2.1±1.3	3.2±1.1 ^c	3.5±1.1	3.7±1.1	3.9±1.1
Pregnancies (total) ^a					
(%/transfer)	0 (0.0%)	13 (30.2%) ^c	116 (43.8%) ^b	76 (54.7%)	43 (63.2%)
Chemical	0 (0.0%)	1 (2.3%)	15 (5.7%)	10 (7.2%)	3 (4.4%)
Ectopic	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.4%)	1 (1.5%)
Clinical ^a	0 (0.0%)	12 (27.9%) ^c	101 (38.1%)	64 (46.0%)	39 (57.4%)
Singleton (%/clinical)	0 (0.0%)	8 (66.7%)	60 (59.4%)	38 (59.4%)	18 (46.1%)
Multiple (%/clinical)	0 (0.0%)	4 (33.3%)	41 (40.6%)	26 (40.6%)	21 (53.8%)
Twins	0	2	24	20	14
Triplets	0	1	14	5	7
Quadruplets	0	1	3	1	0
Viable ^a	0 (0.0%)	10 (23.3%) ^c	85 (32.1%) ^b	53 (38.1%)	36 (52.9%)
Implantation rate ^a	0.0% (0/15)	13.8% (19/138)	17.1% (160/934)	19.0% (98/516)	24.0% (64/267)

^ap<.05, comparing groups 2 through 5; ^bp<.05, comparing groups 4 and 5 to group 3; ^cp<.05, comparing group 2 to groups 3, 4, and 5.

Table 3. — Comparison of pregnancy rates by maximum cell stages of embryos transferred and age - fresh embryo transfers.

	Embryo Development - maximum cell stage in embryo pool transferred				
	4-cell	5-cell	6-cell	7-cell	8-cell
# of transfers	36 (5.3%)	32 (4.7%)	75 (11.1%)	91 (13.5%)	441 (65.3%)
Pregnancies (total) ^a					
(%/transfer)	5 (13.9%) ^c	9 (28.1%) ^d	27 (36.0%) ^c	45 (49.4%)	295 (66.9%)
Clinical ^a	5 (13.9%) ^c	6 (18.7%) ^d	21 (28.0%)	40 (43.9%)	237 (53.7%)
Singleton (%/clinical)	4 (80.0%)	2 (33.3%)	17 (80.9%)	24 (60.0%)	128 (54.0%)
Multiple (%/clinical)	1 (20.0%)	4 (66.7%)	4 (19.1%)	16 (40.0%)	108 (45.6%)
Viable ^c (%/transfer)	4 (11.1%) ^c	6 (18.8%) ^d	20 (26.7%)	36 (39.6%)	210 (47.6%)
Implantation rate ^a	8.5% (7/82) ^c	11.1% (10/90) ^d	10.6% (26/246) ^c	18.4% (59/320) ^b	24.4% (385/1580)

^ap<.05, comparing groups 1 through 5; ^bp<.05, comparing groups 4 to 5; ^cp<.05, comparing group 3 to groups 4 and 5; ^dp<.05, comparing group 2 to groups 3, 4, and 5; ^ep<.05, comparing group 1 to groups 2, 3, 4, and 5.

Table 4. — Comparison of pregnancy rates by maximum cell stages of embryos transferred and age - fresh embryo transfers.

	Embryo Development - maximum cell stage in embryo pool transferred				
	4-cell	5-cell	6-cell	7-cell	8-cell
Patients ≤ 39 years old					
# of transfers	29 (5.0%)	24 (4.1%)	90 (15.5%)	74 (12.7%)	365 (62.7%)
Pregnancies					
Clinical (%/transfer)	5 (17.2%)	6 (25.0%)	40 (44.4%)	34 (45.9%)	209 (57.3%)
Viable (%/transfer)	4 (13.8%)	6 (25.0%)	33 (36.7%)	29 (39.2%)	191 (52.3%)
Implantation rate	9.6% (7/73)	13.7% (10/73)	17.3% (58/336)	23.9% (61/255)	27.5% (345/1254)
Patients ≥ 40 years old					
# of transfers	7 (5.5%)	8 (6.3%)	17 (13.5%)	18 (14.3%)	76 (60.3%)
Pregnancies					
Clinical (%/transfer)	0 (0.0%)	0 (0.0%)	3 (17.6%)	4 (22.2%)	28 (36.8%)
Viable (%/transfer)	0 (0.0%)	0 (0.0%)	3 (17.6%)	3 (16.7%)	19 (25.0%)
Implantation rate	0.0% (0/9)	0.0% (0/17)	11.1% (6/54)	6.5% (5/77)	12.3% (40/326)

were lower for transfers with a maximum cell stage of 6, 5, or 4. The relationship between cell stage and outcome was similar in frozen cycles (data not presented).

Within each embryo group, there was no difference in the mean cell stage of the pool of embryos transferred by pregnancy outcome: 7.1 vs 7.1 in group 5; 5.8 vs 5.7 in group 4; 5 vs 5.1 in group 3; 4.3 vs 4.5 in group 2; and 3.4 vs 3.7 in group 1, pregnant vs non-pregnant, respectively.

The cycles were stratified by age of female at time of transfer (Table 4); 62.7% of younger women and 60.3% of older women had transfers with at least one 8-cell embryo. The same association between cell stage and outcome was noted in both age groups. However, the younger women had higher pregnancy and implantation rates. Small sample size for the cell stage groups in older women prevented reliable statistical inference.

Cycles were stratified by use of ICSI (Table 5). In ICSI

Table 5. — Comparison of pregnancy rates by maximum cell stages of embryos transferred and insemination method - fresh embryo transfers.

	Embryo Development - maximum cell stage in embryo pool transferred				
	4-cell	5-cell	6-cell	7-cell	8-cell
Cycles using ICSI					
# of transfers	18 (6.2%)	13 (4.5%)	40 (13.95)	31 (10.7%)	187 (64.1%)
Pregnancies					
Clinical (%/transfer)	4 (22.2%)	1 (7.7%)	10 (25.0%)	13 (41.9%)	93 (49.7%)
Viable (%/transfer)	3 (16.7%)	1 (7.7%)	10 (25.0%)	11 (35.5%)	83 (44.4%)
Implantation rate	13.3% (6/45)	5.4% (2/37)	9.7% (13/134)	20.8% (22/106)	21.5% (142/660)
Cycles using standard insem.					
# of transfers	18 (4.7%)	19 (4.9%)	35 (9.1%)	60 (15.5%)	254 (65.8%)
Pregnancies					
Clinical (%/transfer)	1 (5.6%)	5 (26.3%)	11 (31.4%)	27 (45.0%)	144 (56.7%)
Viable (%/transfer)	1 (5.6%)	5 (26.3%)	10 (28.6%)	25 (41.7%)	127 (50.0%)
Implantation rate	2.7% (1/37)	15.1% (8/53)	11.6% (13/112)	17.3% (37/214)	26.4% (243/920)

cycles, 64.7% of transfers involved at least one embryo at the 8-cell stage; 65.8% of standard cycles involved at least one 8-cell embryo. These data also demonstrate that the higher the cell stage, the higher the PR and implantation rate. However, small sample size in some of the cell stage groups in cycles with ICSI prevented reliable statistical inference.

Discussion

The majority of embryos that attain blastocysts on day 5 reached an 8-cell stage by day 3 [3]. The explanation frequently given to patients who have no blastocysts to transfer is that even if there had been 3-day-old embryos to transfer, the fact that they did not reach the blastocyst stage indicate that pregnancies would not have occurred had a day 3 transfer been performed. However, the clinical and viable PRs for fresh and frozen ET with at least one 5-7 cell embryo was 34 and 31% for fresh and 38 and 32% for frozen ETs. Thus the possibility exists that in the attempt to get the best PR per transfer with blastocyst transfer, some embryos that may have resulted in pregnancies if they had been transferred at day 3, failed to survive in vitro to blastocyst stage.

There are still many IVF centers that do not have PRs following frozen ET that are comparable to their rates following transfer subsequent to the retrieval. Some IVF centers seem to have lower implantation rates than others even following fresh ET probably related to nuances in embryo laboratory quality and experience and skill. In these situations the IVF center may try to overcome some of these deficits and attain a reasonable PR by transferring more embryos. Sometimes this practice may lead to an unwanted multiple gestation. The transfer of a smaller number of blastocysts would seem ideal for such centers.

However, the possibility exists that the centers with the best implantation rates following transfer on the retrieval cycles, and the best PRs following frozen ET, will also be the ones where 93% of the women having oocyte retrieval will have at least one blastocyst transferred, whereas the groups with lower implantation rates will find 40-50% of the cases having retrieval but no transfer of embryos.

Ideally, no more than two blastocysts should be transferred, though many centers reporting their initial data are transferring more than two in some patients. Though pregnancies have been achieved by transferring frozen-thawed blastocysts, more studies are needed to see how well blastocysts survive the freeze-thaw process especially in comparison to freezing at the 2 pronuclear (2PN) stage.

The most expensive and the riskiest part of the IVF procedure is the use of the medications needed to induce a state of controlled ovarian hyperstimulation (COH), and also the oocyte retrieval process. Frozen ET is innocuous and should not be expensive. Thus one obvious advantage of having a strong cryopreservation program is that the PR is maximized for each oocyte harvest.

Our policy is to allow twice as many embryos to go to day 3 as are to be transferred, place the best half back to the uterus and, then freeze the lesser quality embryos. Thus, some of the frozen-thawed embryos that were transferred were already deselected embryos with a lower cell number. This would partially explain why 65.3% of zygotes reached the 8-cell stage on the retrieval cycle, but only 39.6% attained an 8-cell stage after freeze-thawing. To date there do not appear to be any studies determining the percentage of frozen-thawed zygotes or multi-cell embryos that develop to the blastocyst stage.

Though there was no significant difference in PRs and implantation rates in gr 4 where only one 8-cell embryo was transferred (average cell number 5.8) vs gr 5 with at least two 8-cell embryos (average cell number 7.1), there was at least a trend toward higher rates in gr 5 consistent with the conclusion that higher PRs can be achieved by transferring those embryos that obtain 8-cell states with 7-cell a close second. Thus, a higher PR on the retrieval cycle could probably have been obtained had we allowed more embryos to attain 3-day status and thus transfer more 8-cell embryos. However, we cannot be sure that the PR and implantation rate would be as high on subsequent frozen ET. Though pregnancies occurred following transfer of thawed multi-cell embryos, or even multi-cell embryos that had even been cryopreserved and thawed once before [4], the highest PRs and implantation rates at our IVF center are seen when the embryos are frozen at the 2PN stage.

However, it is not clear if this is a better stage to freeze or whether the lower rates seen with freezing of multi-cell embryos are related to the deselection process [7].

There has been a previous randomized study of ET results after 3 or 5 days of embryo culture which found a PR of 26% on day 3 and 25% on day 5 and implantation rates of 13% and 12%, respectively [8]. The only group that was found to have a higher implantation rate than 8-cell embryos was when the transfer was exclusively of one to two expanding blastocysts. This represented only 25% of the total cavitated embryos and only 14% of the total of non-cavitated embryos and cavitated embryos on day 5 [8]. Another recent study also failed to find any difference in pregnancy rates from day 3 or day 5 transfers [9]. In contrast to the study by Scholtes *et al.* which could be criticized for using a single medium [8], the one by Coskun *et al.* [9] adjusted the medium for blastocysts. The possibility exists that we have maximized the quality of embryos that we can transfer by day 3 but new medias may help improve the percentage of expanding blastocysts from a given pool of fertilized oocytes which could maximize a woman's chance of conception on her first transfer. However, maximizing the PR at first transfer should not occur at the expense of the overall PR achieved per oocyte harvest. One has to consider opportunities to have a second transfer if the first one fails to achieve a successful pregnancy, or for second pregnancies with much less risky and costly frozen ETs rather than going through COH and oocyte retrieval once again. Also the possibility exists that by proper selection criteria one can choose on day 3 embryos that are of the same quality and have the same high implantation rates as the ones manually selected by waiting until day 5 [10].

The centers with the best blastocyst experience need to determine at what cell number by day 3 expanded blastocysts are not likely to develop and these should thus be cryopreserved. The survival rate upon thawing and subsequent PR after transfer would then be needed to be compared to survival rates and subsequent PRs of 2PN cryopreserved thawed embryos that were transferred at similar cell stage number to be sure that the quest for forming blastocysts does not adversely affect subsequent PRs with frozen ET. Furthermore, it will be important to determine the efficacy of freezing-thawing blastocysts in case more than two reach an appropriate level.

It is the authors' belief that those centers having the best success with PRs following transfer of blastocysts should randomly assign patients to 3- or 5-day transfer using comparable groups (our study used an unselected population including women with high serum FSH levels in early follicular phase, poor responders, and women with multiple previously failed cycles), and allow an equal number of fertilized oocytes to attempt to reach an 8-cell stage at 3 days or blastocysts at 5 days. Furthermore, the study should not only compare PRs on first transfer but compare PRs per oocyte harvest thus counting subsequent frozen ETs. We believe that only in this way can we determine whether the quest to maximize the implantation rate on first transfer by transferring bla-

stocysts is not at the expense of an overall sacrifice of PRs per oocyte harvest. Until these data have been provided it may not be justified for centers doing well with day-2 or day-3 transfers to abandon their current practices in favor of the more difficult and expensive attempts to produce blastocysts.

One has to be careful in the interpretation of very high PRs following blastocyst transfer in smaller selected patient groups and extrapolate that all centers transferring blastocysts will have the same outcome. For example, Gardner *et al.* [11], have published to date the highest PRs following blastocyst transfer but that group has traditionally had one of the highest PRs in the world following transfer of day-3 embryos. Randomized comparative studies from groups like these are needed and eagerly awaited.

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