

The Pregnancy Outcomes of Day 2 versus Day 3 Embryo Transfer: A Cross-Sectional Study

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Abstract

Background: The objective was to evaluate whether extending the embryo culture period from 2 to 3 days would yield a more optimal selection of viable embryos, thereby increasing the pregnancy rate.

Materials and Methods: We have retrospectively analyzed pregnancy rates in the patients who had embryo transfer either on day 2 (582 patients) or on day 3 (387 patients) post-insemination over a 10-month period. The relationship between the quality score of day 2 and day 3 embryos and their respective pregnancy rates was also analyzed.

Results: The demographic and clinical characteristics were similar in both groups. Embryos transferred on day 2 or day 3, were similar morphologically & we found no difference in the distribution of grades between patients who became pregnant and those who failed to become pregnant. Pregnancy rates were slightly higher in patients who had embryo transfer on day 3 (40.72%) than patients who had transferred on day 2 (38.96%), but this difference was not significant.

proportion of embryos with 2-3 cells, four cells, and 5-7 cells, which selected for transfer, showed significant difference between day 2 and day 3 ($p < 0.05$). There was also significant difference between pregnant and non-pregnant women based on embryo cell numbers on day two ($p < 0.011$).

Conclusion: Extending the embryo culture period from 2 to 3 days had no adverse effect on pregnancy rate. Embryo transfer could be done on days 2 or 3 according to the convenience of the patient and the medical team.

Keywords: Pregnancy Rate, Day 2 or 3 ET, Embryo Grade, Embryo Cell Number

Introduction

Over the years, pregnancy rates after human embryo transfers have increased from ranges disappointingly low of 18 percent (1) to current rates of 60 percent (2, 3). This significant increase in Assisted Reproductive Technology (ART) outcome is due to improvement in embryo culture and laboratory conditions (4, 5).

However, despite the significant progresses in ART treatments and higher pregnancy rates with transfer of good quality embryos, some couples experience repeated unsuccessful cycles even after two failures. Different etiologies have been proposed. Embryo selection (Viability and good cell divisions) is probably the most important factor for improvement of implantation rate of a given cycle (6). Various strategies such

as transfer of more advanced stages embryos or blastocyst have been suggested to improve results after such implantation failures (7).

Embryo transfer (ET) is traditionally performed two days after oocyte retrieval. Transfer of embryos on day 2 is prior to the activation of the embryonic genome which occurs at the four to eight cell stages (8). Therefore, measurement of embryo quality based on embryonic genome is not possible and this selection is not precise. The embryo selection is difficult especially when more than three embryos exist. Extending the culture period beyond of expected time for the embryonic genome activation (or blastocyst stage) would allow further development of the embryo, might therefore optimize the selection of viable embryos for transfer (9), and might

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have a positive effect on pregnancy outcomes (10).

Transfer on day 3 could eliminate embryos; which have arrested at this sensitive stage and therefore allows selection of more viable embryos for transfer (11). Although more staying in culture environment will lead to more infection and embryo loss, this method might be useful for patients who have experienced repeated failure with day 2 embryo transfers.

Several studies for comparing embryo transfer on day 2 versus day 3 after oocyte retrieval have been performed but the conclusions are conflicting. Some studies have resulted that extending the embryo culture period from 2 to 3 days had no effect on implantation, pregnancy or abortion rates in In Vitro Fertilization/Intra Cytoplasmic Sperm Injection (IVF/ICSI) programs (9, 12). On the other hand, other studies have demonstrated more negative effects of day 3 transferring than day 2. For example, lower rates of pregnancy/ transfer, implantation and birth/ ongoing pregnancy (13) or decreasing of overall quality score in embryos are kept in culture until day 3 (14) have been reported. However, other studies have demonstrated positive effects such as higher implantation (12) or pregnancy rate (15) after transfer on day 3.

The aim of this retrospective study was to examine whether delaying embryo transfer until day 3 allows better discrimination between viable and non-viable embryos, and to assess the effects of longer exposure to culture conditions on embryo development and pregnancy rate. This study has performed for preparing a base for our future prospective study about the results of embryo transfer at blastocyst stage.

Materials and Methods

This is a retrospective study performed at the Royan institute, during the period from September 2005 to June 2006. Day 2 versus day 3 embryo transfers were compared in this study including a total of 969 IVF/ICSI cycles with embryo transfers.

Embryos transferred on day 2 or 3 (grade A, B, or AB, with 4-6 blastomeres on day two, or 6-8 blastomeres on day 3) were considered to be good quality embryos.

Female partners of the couples included in the study were <40 years old. Only transfer cycles in which at least two embryos were available

for transfer were included in the study. All categories of female or male factors infertility except uterine factor infertility were eligible for participation in the study.

Patients who received clomiphene citrate or HMG only protocols were excluded from the study.

In our IVF center, we work 6 day a week with Friday as a holiday. Patients who have oocyte retrieval on Saturday until Tuesday will have their ET after 48h. Patients who have retrieval on Wednesday or Thursday will have ET after 72h to avoid egg retrieval and embryo transfer on Friday.

Ovarian stimulation and oocyte retrieval

Most patients were stimulated with standard GnRH long protocol, which has been published before (16). This protocol began with pituitary desensitization using a GnRH agonist (Lucrin; Abbot, Aubonne, France) in the mid luteal phase of the preceding menstrual cycle (17). Administration of gonadotropins (gonal F; Serone, Aubonne, Switzerland) was initiated on day 3 of the commencing cycle. When the leading follicle reached a diameter of 18 mm, 10000 IU HCG (Pregnyl; Organon) was administered to trigger ovulation. Oocytes were retrieved 36h after HCG injection and were subjected to IVF or ICSI.

Oocyte and embryo culture

Controlled ovarian hyperstimulation, oocyte retrieval, sperm preparation for IVF/ICSI, embryo culture and evaluation performed as described previously (18, 19).

After retrieval, the oocytes were incubated in G1.2 media (Vitrolofe, Motndalswage, Goteborg) under mineral oil in tissue culture dishes (Falcon 3001) at 37°C. The presence of two pronuclei and two polar bodies was assessed 16-18 h after IVF or ICSI. Patients with a yield of at least seven normally fertilized oocytes were selected for transfer on either day 2 or day 3 after oocyte retrieval. Embryos were classified based on morphological criteria that has been described previously (20).

As a rule, at least two embryos were transferred in all patients if two embryos of excellent or good quality were available.

Embryo development and morphology

On the morning of embryo transfer, embryos were examined and the number of cells determined. Each embryo was scored according to its symmetry and the extent of fragmentation of blastomeres (21-23). Briefly, grade A embryos contained symmetrical and unfragmented blastomeres, grade B were even but with slight cellular debris and grade 3 had at least one degenerated cell. Embryos were assigned to grade 4 if three or more cells had completely fragmented, and to grade 5 if all of the blastomeres were degenerated.

Embryo transfer

Embryos with the best morphology and at the most advanced stage of development were selected for transfer. Usually two or more embryos were transferred. A number of factors were taken into consideration when deciding how many embryos to transfer (24), including the patient’s age, cause and history of infertility and the number and grade of the available embryos. Clinical pregnancy was defined as a positive pregnancy test followed by the presence of gestational sac on transvaginal ultrasound 4 weeks after transfer.

Results

A total of 969 patients (581 on day 2 and 388 on day 3) were included in the study. Table 1 shows the demographic and clinical characteristics of the participating women.

The groups did not differ in mean age, duration of infertility, number of previous ART attempts, type of protocol, as well as infertility diagnosis.

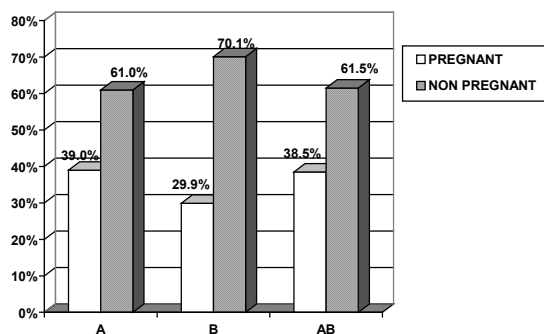


Fig 1: Distribution of embryo grades on day 2 from patients who became pregnant and who failed to become pregnant

The proportion of patients became pregnant varied according to the number of embryos transferred and previous ART attempts. Transfer

of two available embryo resulted in a pregnancy rate of~ 26%, regardless of the timing of the transfer. The highest pregnancy rate (46.7%) resulted from the five embryos transfer, on day 3, whereas the highest pregnancy rate (43.4%) was demonstrated from the 4 embryos transfer on day 2. However, there were no significant differences in the number of transferred embryos between pregnant and non-pregnant women. The patients who had 3 or more trial for ART (IVF failures) showed non significant higher pregnancy rate on day 2 than day 3 (Table 3).

Table 1: Patients characteristics in days 2 and three embryo transfer

	Day 2	Day 3	P value
Number of Cycle-	581	388	
Age (yr)			
18-25	108(18.6%)	60(15.5%)	0.498
26-30	199(34.3%)	130(33.5%)	
31-35	169(29.1%)	127(32.7%)	
36-39	107(17.9%)	71(18.3%)	
- Duration of Infertility (yr)	8.25±5.015	8.43±4.95	0.577
- Infertility diagnosis			
Male factor (%)	329(57.9)	204(53.4)	0.343
Tubal factor	93(16.4)	60(15.7)	
An ovulation (%)	53(9.3)	51(13.4)	
Unexplained (%)	35(6.2)	30(7.9)	
PCO(%)	44(7.7)	30(7.9)	
Endometriosis (%)	14(2.5)	7(1.8)	
- Number of Previous ART attempt			
1	441(76%)	294(76.2%)	0.913
2	93	64(16.6%)	
>=3	46(7.9%)	28(7.3%)	
- Type of protocol			
Long protocol (%)	565(97.2)	346(96.9)	
Short protocol	7(1.2)	6(1.5)	0.577
Antagonist (%)	9(1.5)	6(1.5)	

Outcome of the two ET groups in terms of pregnancy rate is shown in table 2. Although the clinical pregnancy rates per oocyte retrieval (40.82% vs. 39.37% respectively)

and per embryo transfer (40.72% vs. 38.96% respectively) was slightly higher in the day 3 embryo transfer group compared with day 2, this difference was not significant.

Embryo grades, cell number and pregnancy

There were no significant differences in mean number of embryo quality grades (grade A, B, AB), between two groups (Table 2). We also found no difference in the distribution of grades between patients who became pregnant and those who failed to become pregnant (Fig 1, 2).

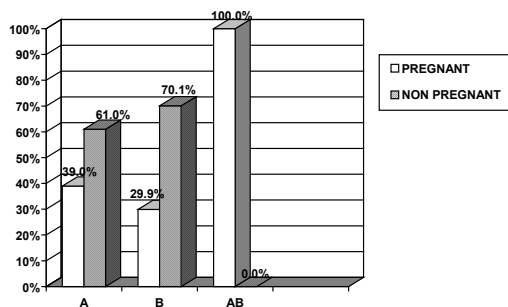


Fig 2: Distribution of embryo grades on day 3 from patients who became pregnant and who failed to become pregnant

Number of embryos with 2-3 cells, four cells, and 5-7 cells, which selected for replacement, showed significant difference between day 2 and day 3 (Table 2). There was significant difference between pregnant and non-pregnant women based on embryo cell numbers on day 2 ($p < 0.011$) (Fig 3).

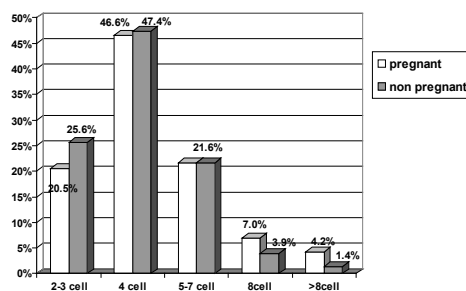


Fig 3: Distribution of embryo cell numbers on day 2 from patients who became pregnant and who failed to become pregnant ($p < 0.05$).

Transferring of embryos with 4-cells showed the highest pregnancy rate (46.6%), whereas 4.2% of patients who had more than eight cells embryos showed the lowest pregnancy rate. On day 3, the lowest pregnancy rate was related to embryos with 2-3 cells (6.7%), but the highest pregnancy rate was shown in eight cells embryos (36.1%). However, there were no significant differences

in distribution of cell numbers between patients who became pregnant and those who failed to become pregnant on day 3 (Fig 4).

Table 2: Reproductive outcome in IVF/ICSI patients having day 2 or day 3 embryo

Transfer	Day 2	Day 3	P Value
- Mean Number of oocyte retrieved	11.18+6.32	9.21+5.18	<0.0001*
- Mean Number of Embryo transferred	3.27+0.808	3.26+0.827	0.724
- Clinical pregnancy rate per ET	38.96%	40.72%	>0.05
- Clinical pregnancy rate per retrieval	39.37%	40.82%	>0.05
Embryology			
Quality based on degree of fragmentation			
Excellent quality embryos (grade A)	16.5+1.39	1.53+1.35	0.189
Good quality embryos (grade B)	0.84+1.05	0.74+0.95	0.14
Moderate quality embryo (grade AB)	0.65+0.93	0.75+1.005	0.086
Number of blastomeres in embryos selected for replacement			
2-3 cell	23.6%(216)	6.5%(41)	
4 cell	47.1%(431)	13.7%(86)	
5-7 cell	21.6%(198)	32.5%(204)	<0.00001*
8 cell	5.1%(47)	34.8%(218)	
>8 cell	2.5%(23)	12.4%(78)	
Mean number of embryos wich completed cleavage stages			
Second cleavage stage (>=4 blastomeres)	90.4(539)	59.8%(382)	<0.00001*
Third cleavage stage (>=8 blastomeres)	9.6%(57)	40.2%(257)	

* significant difference

Distribution of embryos transferred which had completed the second cleavage division (≥ 4 blastomers) and the embryos which proceeded through the third cleavage division (≥ 8 blastomeres) showed significant differences between day 2 and day 3 ($p < 0.0001$) (Table 2). There was also significant difference between second and third cleavage stages in distribution of pregnancy rate ($p < 0.0001$) (Table 3).

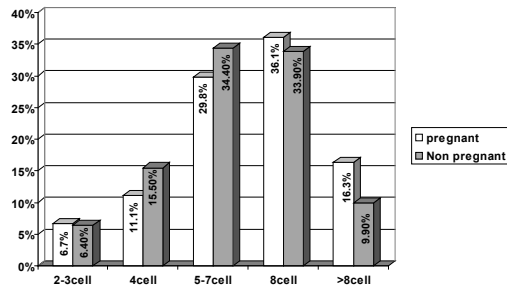


Fig 4: Distribution of embryo cell numbers on day 3 from patients who became pregnant and who failed to become pregnant ($p > 0.05$)

Table 3: Pregnancy rate based on the number of embryo transferred, previous ART attempt and cleavage stages

	Day 2	Day 3	P value
ET			
2	26.2%	25.4%	0.907
3	39.8%	45.8%	0.222
4	43.3%	40%	0.537
≥ 5	40.9%	46.7%	0.729
ART attempt			
1	40.1%	42.2%	0.581
2	32.3%	42.2%	0.204
≥ 3	41.3%	21.4%	0.08
Cleavage stages			
Second stage			
(≥ 4 cell)	58.2%(216)	41.8%(155)	0.094
Third stage			
(≥ 8 cell)	22.6%(33)	77.4%(113)	0.68

* Significant difference

Discussion

In this retrospective study, we have examined pregnancy rates following embryo transfer on day 2 or 3 after oocyte retrieval in a group of 969 patients, who were compared for age, infertility duration, type of treatment protocol, the number of previous treatment cycles, and cause of infertility. Moreover, mean number of transferred embryos was comparable between

groups. There were significant differences about mean number of ovum picked up between two groups ($p < 0.0001$).

Although the pregnancy rate was slightly higher after transfer on day 3 than on day two, based on our all three determinators (Table 2), these differences were not statistically significant.

These results are in agreement with the findings of Edwards et al. (1), and Dawson et al. (11), where the pregnancy rates following transfer on day 3 were higher, but not significantly so, than after transfer on day 2. Huisman et al. (25) in a large retrospective study compared IVF results after day 2 and 3 of ET. The implantation and pregnancy rate was similar in both groups.

Different prospective studies have also demonstrated no significant differences about pregnancy rate between day 2 and day 3 embryo transfers (9, 14, 26).

However, some studies have reported positive effects of embryo transfer on day 3 than day 2 (27, 28). Transfer of embryos to the uterus on day 3 after oocyte retrieval may be closer to the physiological time of arrival of embryo to the uterine cavity than transfer on day 2. Moreover, delaying embryo transfer would allow the selection of the most vital embryos for transfer (14) and these factors may have had positive effects.

Selection of the most viable embryo for transfer is probably the key for a successful IVF program. Most IVF centers use scoring systems mainly based on morphological criteria to select embryos for transfer. Although many factors influence the result of an IVF cycle (e.g. stimulation response, endometrial receptivity, oocyte maturity, culture conditions), embryo morphology is regarded as one of the most important factors (13). Embryo selected for transfer on day 2 and day 3 in our study showed no differences in morphological assessment. In other words, continuing the embryo culture to day 3 had no positive impact on embryo quality. However, in second group, third cleavage stage was more than the first group. Therefore, it seems that if we can delay the time of ET we will able to select better developing embryos. A delay of 1 day may be too short for us to better differentiate the quality of embryos. However, Since human embryonic gene expression only starts from day 3 onwards (8) it is not feasible to predict which embryo will be viable only according to morphological

criteria on day 2 or 3 (29).

Several retrospective studies have investigated this issue. Dawson et al (2) similarly, reported that there is no difference in embryo quality between day 2 and 3 in distribution of embryo grades. However, this result is contrary with some previous studies, which suggested that selection of embryos for transfer based on morphology and development correlates well with pregnancy outcome (30-34).

Delaying embryo transfer until day 3 provides an opportunity to observe the embryos for a further 24 h in culture. Any morphologically normal embryos on day 2 which subsequently arrest or degenerate can be identified and their transfer avoided. This might have a positive effect on implantation rates and future successful pregnancy outcome.

As our retrospective design, we could not measure implantation rate between groups. On the other hand, the data about pregnancy outcome such as early and late abortion, and ectopic pregnancy had not been completely recorded. Therefore, we would not be able to measure these outcomes. A large future randomized clinical trial will be useful for assessment of these variables.

Some authors believe that some suboptimal quality embryos may be rescued in the uterine environment and that extended culture might be a cause of arrest for further development of such embryos (18). So a large proportion of human embryos will arrest *in vitro* between the 4 and the 8-cell stage (35).

The percentage of second cleavage stage on day 2 ET was (90.4%) as compared to the (40.2%) third cleavage stage on day 3 (Table 2). Yet this difference did not improve the pregnancy rate in day 3 over day 2 after we had the opportunity to exclude arrested embryos at 4 and 8-cell stage (Table 3).

It is believed that gene expression of human embryos is switched on around the 8-cell stage immediately before compaction (8). Therefore, nutrient requirements of embryos are different after this stage. Early embryos can grow in a simple salt solution, whereas they require more complex media after they reach the eight-cell stage. These changes also correspond to environmental changes *in vivo* since the embryo reaches the uterus from the fallopian tube at the stage when compaction begins (18). Perhaps

with changing our culture media after day 2, we will gain different results. Further research could explain this hypothesis.

The human cleavage stage embryo normally resides in the oviduct and does not enter the uterus until after compaction (36). The oviduct and uterus provide different nutritional environment for the embryo (2), so it is therefore plausible that premature transfer to the uterus compromises development of cleavage stage embryo. In support of this hypothesis, in other mammalian species, the transfer of cleavage stage embryos to the uterus results in lower pregnancy rates than the transfer of morula or blastocysts (37).

In recent years, therefore, several investigators (11) have tried a more extended delay of embryo transfer, up to blastocyst stage. Extending the culture period to beyond the time of expected activation of the embryonic genome might optimize the selection of viable embryos for transfer (8). In addition, by delaying the embryo culture, embryos with limited and any abnormal developmental potential may be identified and avoided (35). Some chromosomally abnormal embryos fail to develop in culture (38). At present study, the embryo transfer with one day delay not only have no adverse effect on embryo quality and embryo transfer, but also it showed positive effects (non statistically significant) on pregnancy rate. It seems that delaying in embryo transfer until blastocyst stage could improve these results more effectively. Comparison between blastocyst stage and day 2-3 embryo transfer could be checked during a clinical trial.

Conclusion

In conclusion, it seems that there is no difference between day 2 and day 3 embryo transfers, since similar pregnancy rate can be achieved. Moreover, these findings indicate that embryo transfer can be safely scheduled at the convenience of the patient and the centre. Although statistically significant differences between day-2 transfer and day-3 ET were not noted in our study, the number of patients was small, affecting the power of the study. A larger sample is needed to further evaluation of these findings.

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