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A Comparative Analysis Between Day 2 and Day 3 Embryo Transfer in IVF/ICSI: A Retrospective Cross-Sectional Study

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Abstract

Objectives: Although the fertilization and cleavage rate of implanted embryos is about 70%-90% in most patients, only a small number of embryos grown in vitro have the potential to implant. This indicates that many factors are responsible for a successful implantation, including obtaining viable embryos for transfer. This study aimed to examine the clinical results of pregnancy and implantation rates between day 2 and day 3 embryo transfer (ET) in women under the age of 40 experiencing fresh intracytoplasmic sperm injection-embryo transfer (ICSI-ET) cycles.

Materials and Methods: In a retrospective study, a total of 284 ETs were examined from March 2013 to December 2014. The transfer was done according to physician's preference, patient characteristics or number of embryos available.

Results: The data suggested that clinical (35.4% vs. 28.9%, $P=0.26$) or ongoing pregnancy (32.5% vs. 23.7%, $P=0.11$) or implantation rate (0.267 ± 0.2 vs. 0.216, $P=0.09$) was slightly better and the miscarriage rate (3.1% vs. 7%, $P=0.153$) was slightly lower on day 3 ET vs. day 2, however, this difference was not significant. Although most of the baseline characteristics were similar between groups, the number of high-quality embryos (5.29 ± 3.9 vs. 4.47 ± 3.05, $P=0.011$) *a*nd average embryo cleavage score (2.85 ± 0.4 vs. 2.25 ± 0.3, $P<0.001$) was significantly higher in the day 3 ET in comparison to the day 2 ET.

Conclusion: A similar clinical outcome between ET performed on days 2 and 3 in women younger than 40 years undergoing fresh ICSI-ET is suggested by the results of this study.

Keywords: Embryo transfer, Fertilization, Implantation, Pregnancy

Introduction

More than three decades have passed since successful human In Vitro Fertilization (IVF) was started. Although the fertilization and cleavage rate of implanted embryos is about 70%-90% in most patients, the final goal of this procedure, the take home baby rate, is still in the range of 30%-45%. Only 10%-15% of embryos grown in vitro have the potential to implant. This indicates that many factors are responsible for a successful implantation, including obtaining viable embryos for transfer.

Theoretically, an implanted embryo can be received by the uterine cavity in the late morula or early blastocyst stage (4 or 5 days). However, animal studies have shown that the uterus has the ability to accept and maintain the embryo during early cleavage, which can lead to a term pregnancy (1). Many factors affect embryo growth; one is embryo transfer (ET) the day after oocyte retrieval and insemination (2). Studies have found that when the sperm and oocyte are of low quality, ET is more beneficial in the early cleavage stage. Even today, with use of optimal culture media, only 25% of eggs which are fertilized get to the blastocyst stage, but the rest of the embryos (75%) can neither be transferred nor frozen. If these early-stage embryos are frozen before they show signs of degeneration, patients could benefit from a higher number of embryos being transferred, which would result in an increase in the cumulative pregnancy rate (1).

Several studies have compared ET on day 2 vs. day 3 after oocyte recovery, all of which had conflicting results (3-8). For example, although Ertzeid et al (3) found a higher proportion of growth-retarded embryos on day 3; there were morphological similarities between embryos transferred on day 2 and day 3. The increase in the live birth rate from 18.5% to 22.6% on day 3 was not statistically significant (3). Shahine et al (5), while examining poor responders, compared ET on day 2 to day 3 and found no difference in pregnancy outcome, indicating similar options in poor responders, depending on clinician and patient preferences. In a study by Laverge et al (6), no difference was found in implantation and pregnancy rates between transfer on

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day 2 versus day 3; however, the overall quality score of the embryo decreased when the embryos were stored up to day 3. Another prospective study examined whether delaying ET leading up to day 3 would provide a better distinction between viable and non-viable embryos. Although pregnancy rates were not different on day 3 vs. day 2, the rate of implantation was substantially greater after transfer on day 3 (23%), as obtained from the proportion of embryos reaching the fetal heart stage, compared with 19% on day 2. The authors stated that an additional 24 hours of embryo development observation was possible by postponing transfer until day 3 to identify and discard the embryos that are developmentally arrested or retarded (7). Other studies have shown the usefulness of ET in the poor responders on day 2 compared to day 3. There have been more clinical and ongoing pregnancy rates after ET on day 2 than on day 3 in poor responders, suggesting that the occurrence of miscarriage can be reduced by restricting embryo culture to only 2 days which could also provide an alternative for managing poorly responding patients (9,10).

Despite numerous studies researching improvement in ET outcome, optimal management remains a challenge. In this retrospective study, we compared clinical outcomes of rates of pregnancy and implantation between transfer on day 2 and on day 3.

Materials and Methods

This study was a retrospective cross-sectional study performed on infertile patients attending Infertility Clinic in Tehran, Iran, from March 2013 to December 2014. No written/verbal informed consent was provided from the patients. They underwent IVF or ICSI according to the standard protocols. Information collected from patients included demographic, clinical and laboratory data. The subjects were women younger than 40 undergoing intracytoplasmic sperm injection (ICSI) ET cycles (fresh cycle) and had normal endometrial thickness (7-12 mm) on ET day, no visible endometrial pathology and less than 3 failed previous cycles. Patients who had an abnormal uterine cavity as observed on hysterosalpingography or hysteroscopy or more than 3 previous failures of ICSI-ET cycles were omitted from this study. The cause of infertility, patient age and levels of follicle stimulating hormone (FSH) and anti-Müllerian hormone (AMH) on the third day of menstruation were recorded in the questionnaire.

All patients had previously undergone a protocol of ovarian stimulation—long luteal gonadotropin-releasing hormone (GnRH agonist) and antagonist. Details of the protocols have been previously described (11). Briefly, controlled ovarian hyper stimulation was done with either pituitary down regulation in the late luteal phase with a GnRH agonist (Buserelin, 500 mcg/day, Sanofi-aventis Pharmaceuticals, Canada) or mid-follicular pituitary down regulation with a GnRH antagonist (Cetrorelix, 250 mcg/day; Merck Serono, Germany). 150-300 IU/L of recombinant FSH (Gonal-F, 75 IU/L, Merck SeronoInc, Germany, MA) were used to induce multiple follicle growth and stimulation was evaluated with serial ultrasound monitoring. When 2 or more follicles reached a size of 17 mm or more, 10 000 IU/mL of human chorionic gonadotropin (Choriomon, 5000 IU/L, IBSA Inc, Switzerland) was injected and oocytes were retrieved 34-36 hours later through transvaginal ultrasound under anesthesia.

Denudation of the oocytes was performed both mechanically and enzymatically. Fertilization of oocytes and ET was performed according to the protocol previously reported (5). Briefly, after examination of the oocytes for fertilization status, 16-18 hours post insemination, the two pronuclei zygotes were cultured for 24-48 hours in Sage Cleavage Medium (Cooper Surgical, Inc, Trumbull, CT) supplemented with 10% Serum Protein Substitute (SPS, Irvine Scientific, Santa Ana, CA). The embryo culture conditions or techniques remained unchanged in the duration of the study. Two or three embryos at the 4-8 cell stage were transferred based on the patient prognosis (FSH level, age and previous unsuccessful attempts) on the second or third day after insemination. ET took place via soft catheter (Cook Ob/Gyn, Spencer, India) on day 2 or 3 after oocyte recovery. Luteal support was started on the day of ET and patients received vaginal progesterone (Cyclogest, Actover, UK), 400 mg every 12 hours. A positive pregnancy test was confirmed by measuring β-HCG levels above 25 mIU/mL, 15 days after ET. Luteal support was sustained for 12 weeks of pregnancy in the case of a positive pregnancy test.

The number and grade of the embryos' blastomeres were recorded. Embryos of adequate quality were defined as grade 1-2, including a 4-cell embryo on day 2 (two days following oocyte retrieval) and 6 cells on day 3 (three days following oocyte retrieval). Embryos with normal fertilization and grade 1-2 on day 2 or 3 were chosen for transfer. A chemical pregnancy was designated as a positive pregnancy test without the existence of a gestational sac afterwards. In cases with a positive pregnancy test, vaginal ultrasounds were performed 3-4 weeks later, to confirm the existence of a gestational sac and clinical pregnancy. Spontaneous abortion was defined as a clinical pregnancy loss before 24 weeks gestational age. Multiple pregnancy was defined as 2 or more gestational sacs that were shown on ultrasound during pregnancy. Characteristics of patients such as age, body mass index (BMI), basal FSH and AMH levels, prior failed attempts, infertility duration and etiology, the average number of eggs retrieved and the average number of embryos transferred were assessed. The BMI was estimated utilizing the weight/height^2 formula. The women were grouped into three categories: 18.5–24.9 kg/m^2 (normal), 25–29.9 kg/m^2 (overweight) and ≥30 kg/m^2 (obese) (12).

Statistical Analysis

Each group required a minimum of 154 patients to determine an increase in clinical pregnancy rates from 15% to 30% with 80% power and 0.05 alpha errors. The logistic regression of the outcomes stratified by number of oocytes (<5 and ≥5). The two groups were compared with

student's *t* test and chi-square test for different variables including baseline characteristics and outcomes as suitable. A *P* value of <0.05 was assumed to be statistically significant. Data analysis was performed with SPSS 21 software. Confounding variables included day 3 FSH, AMH, number of eggs retrieved, number of embryos transferred, number of attempts, embryo quality score, embryo cleavage score and number of follicles on the day of HCG testing that adjusted for the logistic regression. The primary outcome measure is the clinical pregnancy rate and secondary outcome measures are chemical and ongoing pregnancy, implantation rate and miscarriage rate.

Results

A total of 300 patients younger than 40 years, with a history of successfully generating oocytes and embryos, were registered in this study. Sixteen participants were excluded as a result of insufficient data or the ET being done on day 4 or 5. Finally, 284 women were investigated who underwent ET on day 2 or 3. One hundred fourteen patients underwent transfer on day 2 and 170 underwent transfer on day 3. There was no randomization for transfer days and transfer was done according to physician preference, patient characteristics or number of embryos available. Baseline characteristics of patients and ICSI cycles are shown in Tables 1 and 2.

In order to assess whether the day of ET was an important predictor of pregnancy, a logistic regression was conducted. The regression model was modified with age, FSH, AMH, total number of oocytes retrieved, previous unsuccessful attempts, number of embryos transferred, average embryo quality and cleavage score on day 2 or 3 (Tables 1 and 2). The logistic regression model failed to demonstrate a predictive factor for outcome in this study due to the percentage of the predictive value for ongoing pregnancy rate (approximately 11%) being lower than the standard (40%), (data not shown).

To prevent the effects of poor responders on the clinical pregnancy outcomes, the number of eggs retrieved were divided into the above and below the 5, but no differences were observed in clinical outcomes among patients with poor response owing to earlier day of transfer (day 2), (Table 2).

The results of the present study demonstrated a similar clinical outcome between ET performed on days 2 and 3 in women younger than 40 years undergoing ICSI-ET cycles. The data suggests that clinical (35.4% vs. 28.9%, $P=0.26$) or ongoing pregnancy (32.5% vs. 23.7%, $P=0.11$) or implantation rate (0.267 ± 0.2 vs. 0.216, $P=0.09$) was slightly better and the miscarriage rate (3.1% vs. 7%,

Table 1. Baseline Characteristics of Patients Undergoing ICSI-ET: Day 3 vs. day 2 ET

Variables	Day 2 ET (n = 114)	Day 3 ET (n = 170)	df	Tests	*P* value[a]
Average age (y)	32.7 ± 5.94	32.45 ± 5.98	282	t = 0.38	0.70
BMI (kg/m^2)	27.66 ± 5.29	27.72 ± 4.10			0.92
Day 3 FSH (mIU/mL)	5.83 ± 2.25	5.95 ± 2.57	271	t = -0.38	0.69
AMH (ng/mL)	3.65 ± 3.35	3.67 ± 3.87	254	t = -0.02	0.97
Infertility duration (y)	7.41 ± 5.46	5.13± 6.68	227.20	t = 1.12	0.26
Attempt no.	1.34 ± 0.77	1.40 ± 0.76	281	t = -0.64	0.51
BMI (kg/m^2)			2	χ^2 = 3.52	0.17
Normal and underweight (<24.9)	32 (31.1)	36 (22.8)			
Overweight (25-29.9)	40 (38.8)	79 (50)			
Obese (≥30)	31 (30.1)	43 (27.2)			
Infertility etiology			3	χ^2 = 1.14	0.76
Male factor	56 (49.6%)	81 (47.7%)			
Tubal factor	12 (10.6%)	13 (7.6%)			
DOR	12 (10.6%)	19 (11.2%)			
Multiple factors	31 (29.2%)	57 (33.5%)			

Abbreviations: ET, Embryo Transfer; BMI, body mass index; FSH, Follicle stimulating hormone; AMH, anti-Müllerian hormone (AMH); DOR, diminished ovarian reserve.

[a] *P* value < 0.05 significant

Table 2. Baseline Characteristics of ICSI Cycles: Day 3 vs. Day 2 ET

Variables	Day 2 ET (n = 114)	Day 3 ET (n = 170)	df	Tests	P value[a]
Average high quality embryos	4.47 ± 3.05	5.29 ± 3.99			0.011
Average embryo quality score	2.31 ± 0.58	2.31 ± 0.59	277	t = 0.016	0.98
Average embryo cleavage score	2.25 ± 0.37	2.85 ± 0.46	277	t = -11.562	<0.001
Average no of embryo transferred	2.95 ± 0.82	2.88 ± 0.85	282	t = 0.66	0.50
Gonadotropin ampules of 75 IU FSH	32.50 ± 12.72	33.11 ± 12.84	281	t = 0.89	0.37
Average egg no. retrieved (%)			1	χ^2 = 2.33	0.33
No < 5	20 (17.5%)	19 (11.2%)			
No ≥ 5	94 (82.5%)	151 (88.8%)			

Abbreviations: ET, Embryo Transfer; FSH, Follicle stimulating hormone; ICSI, intracytoplasmic sperm injection-embryo.

[a] *P* value < 0.05 significant

$P=0.153$) was slightly lower, on day 3 ET vs. day 2, but this difference was not significant. Although most of the baseline characteristics were similar between both groups, the number of high-quality embryos (5.29 ± 3.9 vs. 4.47 ± 3.05, $P=0.011$) and average embryo cleavage score (2.85 ± 0.4 vs. 2.25 ± 0.3, $P=0.001$) was significantly higher in the day-3 ET as compared to the day-2 ET (Table 3).

Discussion

Previous research has reported that the implantation of the embryos on day 1 was comparable to days 2 and 3 (13-15). Quinn et al (16) stated that the culture environment used for the gametes influenced pregnancy rate. That is, under suboptimal laboratory conditions, pregnancy rates can be improved by earlier ET, which was not a factor in the present study. The authors mentioned that if culture conditions are optimized, no differences would be seen in pregnancy rate after ET on day 1or day 2. In other studies, embryos transferred on day 2 were comparable to day 3 (5,6,17-20). Although in these studies, in patients with good prognosis, fewer embryos were transferred, in our survey, between the two groups, the number of embryos transferred remained unchanged.

Many studies also compared day 2 with day 3 ETs, but there seems to be no consensus (4,5,21,22). For example, Laverge et al (6) observed similar clinical pregnancy and implantation rates between days 2 and 3, while overall embryo quality scores were lower on day 3. However, in this study, only a short agonist stimulation protocol was used, which could affect results on oocytes and embryo quality (23) that were different from our study using agonist and antagonist protocols for ovarian stimulation. Shen et al (9) found that ET on day 2 enhanced rates of ongoing pregnancy and decreased the rates of miscarriage in cycles with lower numbers of embryos in patients with poor response younger than age 40 years. However, there was no difference in patients older than 40 years in these outcomes.

The authors also found that miscarriage rates on day 2 were lower than day 3 transfers, which could account for the higher rates of ongoing pregnancy for transfer day 2 in this study. However, this study was conducted in 2 different periods, and on two age ranges; thus, unknown factors may also affect clinical outcomes, which may be different with our study. This group also transferred lower numbers of embryos in patients with a good prognosis; however, in our study, the patients were younger than 40 years.

We found no benefits in clinical outcomes regarding pregnancy and miscarriage rates among patients with poor response by transfer on day 2, even after dividing into groups by the number of recovered oocytes under and above 5. Multiple pregnancy rates between ET on day 2 or day 3 (33.3% vs. 16.2%, $P=0.12$) were the same. The obtained data was in line with that performed by Shen et al (9), (34.5% vs. 37.5%, NS), stating that the observed reason may be due to the higher number of embryos transferred in poor responders in their study. Bahceci et al (10) observed that clinical pregnancy rate per transfer was significantly higher in transfers on day 2 vs. day 3 in poor responders, but there was no difference in implantation rates, as in our study.

A Cochrane meta-analysis failed to demonstrate that later ET from day 2 to 3, caused improvement in the live birth rates. Clinical pregnancy rates can be improved by ET on day 3, but due to a higher miscarriage rate with the day 3 ET, the live birth rate remained the same (5). However, in another study it was suggested that the embryo selection occurs during the cleavage period. Therefore, a delay in ET to day 3 may lead to selection of better quality embryos for transfer (22).

In a retrospective study, Dawson et al (7) showed that the pregnancy rate was higher with day 3 ET, but this was not statistically significant (35% vs. 31%, NS); however, implantation rates showed a significant difference (23% vs. 19%, $P<0.05$) and the miscarriage rate was lower on day 3(6% vs. 2%, $P<0.05$). The authors suggested that postponing ET from day 2 to day 3, 16% of embryos stopped growing. Waiting until day 3 allowed us to identify these

Table 3. Clinical Outcome Between Day 3 vs. day 2 ET in Patients Undergoing ICSI-ET Cycles

Variables		Day 2 ET (n=114)	Day 3 ET (n=170)	Total	df	Chi 2	*P* value*
		No. (%)	No. (%)	No. (%)			
Chemical pregnancy	Positive	4 (3.5)	5 (3)	9 (3.2)	1	0.045	0.83[a]
	Negative	110 (96.5)	159 (97)				
Clinical pregnancy	Positive	33 (28.9)	58 (35.4)	91 (32.7)	1	1.25	0.26[c]
	Negative	81 (71.1)	106 (64.6)	187 (67.3)			
Ongoing pregnancy	Positive	27 (23.7)	53 (32.5)	80 (28.9)	1	2.54	0.11[c]
	Negative	87 (76.3)	110 (67.5)	197 (71.1)			
Miscarriage[c]	Positive	8 (7)	5 (3.1)	13 (4.7)	1	2.34	0.153
	Negative	106 (93)	158 (96.9)	264 (95.3)			
Single pregnancy[c]		27 (81.8)	38 (66.7)	65 (72.2)	1	2.39	0.12
Multiple pregnancy		6 (16.2)	19 (33.3)	25 (27.8)			
		Mean (SD)	Mean (SD)	df	t	95% CI	*P* value*
Implantation rate		0.12 (0.216)	0.17 (0.267)	268.61	-1.76	-0.10-0.006	0.09[b]
Fertilization rate		67.8 (81.10)	64.7 (22.44)	279	0.46	-9.93-16.11	0.64[d]

Abbreviations: ET, Embryo Transfer; ICSI, intracytoplasmic sperm injection-embryo.
*P value < 0.05 significant; [a] Fisher exact test; [b] Mann–Whitney U test; [c] chi-square test; [d] independent t test.

growth-arrested embryos and avoid their transfer.

In our study, the miscarriage rate was higher in ET on day 2, but these differences were not significant (7% vs. 3.1%, $P=0.153$). Perhaps the difference between our study and Dawson et al could be due to selecting patients with poor response. If the poor response rate is low, the miscarriage rate will be low. Under ideal conditions, the culture media could have affected the developing embryos and led to a higher miscarriage rate. In our study, the embryo quality score was the same for both groups, which may be due to the recent developments in culture media and laboratory conditions. Carrillo et al (21) have shown that pregnancy and implantation rates were higher on day 3 than on day 2; however, in contrast to ours and previous studies, in that study, glucose- and phosphate-free culture media were employed. The authors concluded that preservation of the human embryo in a glucose- and phosphate-free environment for an extra 24 hours could better replicate the optimal fallopian tube environment following ovulation which would lead to improved embryo development and higher rates of implantation and pregnancy.

Many studies have shown that in vitro cultured blastocysts have higher implantation rates,which are related to its specific culture media and laboratory conditions and may therefore be more effective in patients who produce an adequate number of high quality embryos at the cleavage stage, facilitating the selection of the best quality embryos (24-26).

Park et al (27) compared culture in a closed system (time-lapse imaging [TLI] incubators) up to 2 days after microinjection with conventional incubation system in IVF cycles. They found no significant differences in clinical pregnancy and implantation rates on ET on day 2 among the two groups. In that study, fixed pictures were only used for evaluation and the extra information provided by TLI was not used in selecting embryos for transfer. In addition, they found that the miscarriage rate in the TLI group was higher. This could be due to the fact that embryo scoring based on traditional criteria is harder in the TLI compared with a high resolution inverted microscope ergo, affecting the selection of embryos for transfer undesirably. In comparison to the standard inverted microscope, the images on the TLI were not clear and the focusing levels were restricted.

In another study, Ahlstrom et al (28) found that conventional morphology predicts better live birth than morphokinetics (produced by TLI) after day 2 transfer. Dar et al (29) found a considerably higher risk of preterm delivery (<37 weeks) in singletons after blastocyst culture compared with cleavage stage (day 3) transfer. They proposed that extended embryo culture may have harmful effects on the resulting placentation.

The retrospective design of the study as well as the unselected population are considered restrictions which may have affected the outcomes. We found no benefits in clinical outcome among patients with poor response after transfer on day 2, even after dividing the number of oocytes retrieval under and above the five groups.

Conclusion

Considering the results of this and earlier studies, ET on days 2 and 3 could be considered similar and acceptable as treatment options for patients with poor and normal response depending on the preference of the physician and the patients' characteristics. Further studies are suggested to demonstrate the potential effects of embryo culture on the embryos and its epigenetic effects on children in later life.

Ethical Issues

This study was approved by the Ethics Committee on Infertility and Reproductive Health Research Center (IRHRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran (SBMU1.REC.1394.33).This was a retrospective cross sectional study and no written/verbal informed consent was provided from the women who underwent IVF/ICSI according to standard protocols.

Conflict of Interests

Authors declare that there is no conflict of interests.

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References

1. Mangoli R, Mangoli V. Advantages and Disadvantages of Embryo Transfer on Day 2, Day 3 and Blastocyst Stage. In: Rao KA, Carp H, eds. Principles and Practice of Assisted Reproductive Technology. India: Jaypee Brothers Medical Publishers; 2014:1010-1016.
2. Knobil E, Neill JD. The Physiology of Reproduction. 2nd ed. New York: Raven Press; 1994:123-188.
3. Ertzeid G, Dale P, Tanbo T, Storeng R, Kjekshus E, Åbyholm T. Clinical outcome of day 2 versus day 3 embryo transfer using serum-free culture media: a prospective randomized study. J Assist Reprod Genet. 1999;16(10):529-34. doi: 10.1023/A:1020597104326.
4. Suzuki T, Shibahara H, Hirano Y, Ohno A, Takamizawa S, Suzuki M. Randomized study comparing day 2 versus day 3 elective transfer of two good-quality embryos. Reprod Med Biol. 2004;3(2):99-104. doi: 10.1111/j.1447-0578.2004.00056.x.
5. Shahine LK, Milki AA, Westphal LM, Baker VL, Behr B, Lathi RB. Day 2 versus day 3 embryo transfer in poor responders: a prospective randomized trial. Fertil Steril. 2011;95(1):330-332. doi:10.1016/j.fertnstert.2010.06.093.
6. Laverge H, De Sutter P, Van der Elst J, Dhont MA. A prospective, randomized study comparing day 2 and day 3 embryo transfer in human IVF. Hum Reprod. 2001;16(3):476-80. doi:10.1093/humrep/16.3.476.
7. Dawson KJ, Conaghan J, Ostera GR, Winston RM, Hardy K. Delaying transfer to the third day post-insemination, to select non-arrested embryos, increases development to the fetal heart stage. Hum Reprod. 1995;10(1):177-182. doi:10.1093/humrep/10.1.177.

8. Fang C, Huang R, Li TT, Jia L, Li LL, Liang XY. Day-2 and day-3 sequential transfer improves pregnancy rate in patients with repeated IVF–embryo transfer failure: a retrospective case–control study. Reprod Biomed Online. 2013;26(1):30-35. doi:10.1016/j.rbmo.2012.10.004.
9. Shen S, Rosen MP, Dobson AT, Fujimoto VY, McCulloch CE, Cedars MI. Day 2 transfer improves pregnancy outcome in in vitro fertilization cycles with few available embryos. Fertil Steril. 2006;86(1):44-50. doi: 10.1016/j.fertnstert.2005.12.020.
10. Bahceci M, Ulug U, Ciray HN, Akman MA, Erden HF. Efficiency of changing the embryo transfer time from day 3 to day 2 among women with poor ovarian response: a prospective randomized trial. Fertil Steril. 2006;86(1):81-85. doi:10.1016/j.fertnstert.2005.12.029.
11. Dayal MB, Frankfurter D, Athanasiadis I, Peak D, Dubey A, Gindoff PR. Day 2 embryo transfer (ET) and day 3 ET afford similar reproductive outcomes in the poor responder. Fertil Steril 2011;95(3):1130-1132. doi:10.1016/j.fertnstert.2010.10.010.
12. WHO. Obesity: preventing and managing the global epidemic. Geneva: WHO; 2000.
13. Scott LA, Smith S. The successful use of pronuclear embryo transfers the day following oocyte retrieval. Hum Reprod 1998;13(4):1003-1013. doi:10.1093/humrep/13.4.1003.
14. Jaroudi K, Al-Hassan S, Sieck U, Al-Sufyan H, Al-Kabra M, Coskun S. Zygote transfer on day 1 versus cleavage stage embryo transfer on day 3: a prospective randomized trial. Hum Reprod. 2004;19(3):645-8. doi:10.1093/humrep/deh125.
15. Dale B, Fiorentino A, de Simone ML, et al. Zygote versus embryo transfer: a prospective randomized multicenter trial. J Assist Reprod Genet. 2002;19(10):456-461. doi:10.1023/A:1020354318164.
16. Quinn P, Stone B, Marrs R. Suboptimal laboratory conditions can affect pregnancy outcome after embryo transfer on day 1 or 2 after insemination in vitro. Fertil Steril. 1990;53(1):168-170. doi:10.1016/S0015-0282(16)53236-1.
17. Gunby JL, Daya S, Olive D, Brown J. Day three versus day two embryo transfer following in vitro fertilization or intracytoplasmic sperm injection. Cochrane Database Syst Rev. 2004;2. doi:10.1002/14651858.CD004378.
18. Aboulghar MM, Aboulghar MA, Mansour RT, Serour GI, Amin YM, Abou-Setta AM. Pregnancy rate is not improved by delaying embryo transfer from days 2 to 3. Eur J Obstet Gynecol Reprod Biol. 2003;107(2):176-179. doi:10.1016/S0301-2115(02)00400-1.
19. Baruffi R, Mauri AL, Petersen C, Felipe V, Franco JJ. Day 2 vs. day 3 embryo transfer after intracytoplasmic sperm injection. A prospective, randomized study. J Reprod Med 2003;48(8):631-634.
20. De los Santos M, Mercader A, Galan A, Albert C, Romero J, Pellicer A. Implantation rates after two, three, or five days of embryo culture. Placenta. 2003;24:S13-S19. doi:10.1016/S0143-4004(03)00172-3.
21. Carrillo AJ, Lane B, Pridham DD, et al. Improved clinical outcomes for in vitro fertilization with delay of embryo transfer from 48 to 72 hours after oocyte retrieval: use of glucose-and phosphate-free media. Fertil Steril. 1998;69(2):329-334. doi:10.1016/S0015-0282(97)00499-8.
22. Dobson AT, Raja R, Abeyta MJ, et al. The unique transcriptome through day 3 of human preimplantation development. Hum Mol Genet. 2004;13(14):1461-1470. doi:10.1093/hmg/ddh157.
23. Loumaye E, Vankrieken L, Depreester S, Psalti I, de Cooman S, Thomas K. Hormonal changes induced by short-term administration of gonadotropin-releasing hormone agonist during ovarian hyperstimulation for in vitro fertilization and their consequences for embryo development. Fertil Steril. 1989;51(1):105-111.
24. Kaur P, Swarankar M, Maheshwari M, Acharya V. A comparative study between cleavage stage embryo transfer at day 3 and blastocyst stage transfer at day 5 in in-vitro fertilization/intra-cytoplasmic sperm injection on clinical pregnancy rates. J Hum Reprod Sci. 2014;7(3):194. doi:10.4103/0974-1208.142481.
25. Beesley R, Robinson R, Propst A, Arthur N, Retzloff M. Impact of day 3 or day 5 embryo transfer on pregnancy rates and multiple gestations. Fertil Steril. 2009;91(5):1717-1720. doi:10.1016/j.fertnstert.2008.02.003.
26. Van der Auwera I, Debrock S, Spiessens C, et al. A prospective randomized study: day 2 versus day 5 embryo transfer. Hum Reprod. 2002;17(6):1507-12. doi:10.1093/humrep/17.6.1507.
27. Park H, Bergh C, Selleskog U, Thurin-Kjellberg A, Lundin K. No benefit of culturing embryos in a closed system compared with a conventional incubator in terms of number of good quality embryos: results from an RCT. Hum Reprod 2014:deu316. doi:10.1093/humrep/deu316.
28. Ahlstrom A, Park H, Bergh C, Selleskog U, Lundin K. Conventional morphology performs better than morphokinetics for prediction of live birth after day 2 transfer. Reprod Biomed Online. 2016. doi:10.1016/j.rbmo.2016.03.008.
29. Dar S, Librach C, Gunby J, Bissonnette F, Cowan L. Increased risk of preterm birth in singleton pregnancies after blastocyst versus Day 3 embryo transfer: Canadian ART Register (CARTR) analysis. Hum Reprod 2013;28(4):924-8. doi:10.1093/humrep/des448.