The Optimal Time for Embryo Transfer in Fresh IVF: Comparison between Day 3 and Day 5 on Pregnancy Outcomes

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체외수정술 후 난할단계 배아와 포배기단계 배아를 이식했을 때의 임신예후의 비교

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목 적: 본 연구는 체외수정술 후 배양 3일째 2개의 난할단계 배아 (D3ET)와 배양 5일째 2개의 포배기단계 배아 (D5ET)를 이식했을 때 각각의 임신예후를 비교하기 위하여 시행하였다.

연구방법: 2007년 1월부터 2009년 6월까지 강남차병원 여성의학연구소에서 체외수정술 후 D3ET을 시행한 90명의 환자를 나이와 체외수정 주기의 특성을 고려하여 D5ET군 90명과 비교한 후향적 환자군-대조군 연구를 시행하였으며, 두 군 모두 2개씩의 양질의 배아를 이식하였다. 각각의 임신율, 착상률, 다태임신율을 비교하였다.

결 과: 환자의 특성, 체외수정주기 및 배아의 특징은 두 군 간에 차이를 보이지 않았다. 임신예후를 비교했을 때, D3ET군과 D5ET군 모두 유의한 차이를 보이지 않았다: 착상률 (39.4% vs. 32.8%), 임신율 (57.8% vs. 46.7%), 임상적임신율 (53.3% vs. 45.6%), 진행임신율 (50.0% vs. 42.2%), 유산율 (13.5% vs. 9.5%). 두 군 모두 높은 다태임신율을 보여주었다 (37.5% vs. 34.1%).

결 론: 체외수정술 후 배양 5일째 포배기단계 배아이식이 배양 3일째 난할단계 이식보다 더 좋은 임신예후를 보여주지 못한다. 또한 나이가 젊고, 양질의 배아를 가진 좋은 예후를 예측할 수 있는 여성에서는 다태임신율을 줄이기 위해 단일배아이식을 고려해야 한다. **[Korean. J. Reprod. Med. 2010; 37(2): 143-151.]**

중심단어: 배아이식, 난할단계이식, 포배기단계이식, 임신예후

The aim of assisted reproductive technologies (ART) including *in vitro* fertilization (IVF) is to get satisfactory pregnancy rate and at the same time to decrease multiple gestations. During ART, the decision of embryo transfer (ET) day has been considered as an important factor. Several studies have suggested that in selected patients, blastocyst-stage ET could get favorable pregnancy outcomes than cleavage-stage ET.[1,2] Day 5 (blastocyst-stage) ET is more physiologic than day 3 (cleavage-stage) ET, because embryos go through the fallopian

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tubes and do not reach the uterine cavity before the morula stage in human.[3] The uterine pulsatility is also significantly reduced at the time when blastocysts are transferred and therefore the possibility of embryos expelling could be decreased.[4] It is regarded that only the most viable embryos are expected to develop into blastocyst. As a consequence of this self selection process, the better quality embryos are selected, which could result in higher implantation rates and favorable pregnancy outcomes.

Day 5 ET, however, is related to a higher incidence of IVF cycle cancellation due to failed embryo development [5,6] and fewer embryos cryopreserved.[2,6] Also, the increased risk of monozygotic twining[7~9] and epigenetic modification associated with extended culture to 5 day should be considered.[10,11] These facts actually could make the patients be distressed. We should consider that day 5 ET is associated with an increased workload, cost, and resources.[12] To apply day 5 ET to each ART cycle, infertility center should have a policy that is able to select the patients who could benefit from the extension of culture day 5, without possibility of being cancelled due to developmental arrest. The aim of this study was to investigate whether day 5 ET was really superior to day 3 ET of equal numbers on pregnancy outcomes.

MATERIALS AND METHODS

1. Patient population

This study was a retrospective matched case control study. 180 patients (day 3 ET group: n=90, day 5 ET group: n=90) treated by IVF at the Fertility Center of CHA Gangnam Medical Center of CHA University were included from January 2007 to June 2009. Subjects were matched for reproductive profiles and cycle characteristics. This study excluded oocytes donation cycles and preimplantation genetic diagnosis (PGD). Equal numbers (n=2) of good quality embryos were transferred in each group.

2. Ovarian stimulation, oocyte retrieval, embryo culture, and embryo transfer

All patients underwent either the GnRH agonist (Lucrine®, Abbott, Rungis, France) or GnRH antagonist (Cetrotide®, Serono, Geneva, Switzerland) protocol for controlled ovarian stimulation (COS) along with daily injections of rFSH (Gonal-F®, Serono or Puregon®, Organon, Oss, Netherlands). After proper pituitary regulation and desensitization using GnRH agonist during the previous midluteal phase, COS was initiated at the beginning of menstrual cycle day 3. In the GnRH antagonist protocol, COS was initiated on day 3 using daily injections of rFSH, with GnRH antagonist added on day 5 or 6, depending on the presence of a 12~13 mm ovarian follicle. The daily dose of rFSH was adjusted for each individual according to her serum estradiol (E_2) concentration, and follicular growth and numbers were assessed by transvaginal ultrasound. Final oocyte maturation was induced by intramuscular administration of 10,000 IU of hCG (Pregnyl®, Organon) when the mean diameter of at least two follicles reached >18 mm on transvaginal ultrasound.

Oocyte retrieval was performed under transvaginal ultrasound-guided aspiration after 36 hours of hCG injection. Retrieved oocytes were fertilized 3~6 hours later, either by conventional insemination or by intracytoplasmic sperm injection (ICSI), depending on the presence or absence of male factor infertility, in IVF medium (Quinn's Advantage media®, Sage BioPharma, Bedminster, NJ, USA). Twenty-four hours after oocyte retrieval, normal fertilization was confirmed by the presence of two pronuclei (2PN) with two distinct or fragmented polar bodies.

Embryos were cultured in culture medium (Sage sequential media®, Sage BioPharma) for 3 days after fertilization. On the morning of day 3, embryos were

transferred from cleavage medium to blastocyst medium. Embryo quality on day 3 of embryo culture was measured according to the following parameter: number of blastomeres, rate of fragmentation, and multinucleation of the blastomeres. Good quality embryos on culture day 3 were defined as having a minimum of 6 blastomeres of normal size, a maximum of 20% of anucleated fragments, and no multinucleated blastomeres. Embryo quality on day 5 assessed according to the criteria of Gardner and Schoolcraft.[13] Good quality embryos on culture day 5 were defined as having blastocoel being equal to or greater than half the volume of the embryo and good inner cell mass and trophectoderm. We counseled patients about the potential risks and benefits of both day 3 ET and day 5 ET. These patients selected ET day based on physician counseling and patient preference. Two good quality embryos were transferred in both groups. Beginning on the day of oocyte retrieval, all patients received daily progesterone support (IMP 50 mg or 600 mg of transvaginal micronized progesterone).

3. Pregnancy confirmation and outcome measures

To assess IVF treatment outcomes, serum β-hCG concentrations were measured 12 days after ET, with a >20 IU/mL increase in serum β-hCG regarded as positive for pregnancy. Serum β-hCG concentrations were measured thereafter in women who showed this increase after 12 days. Clinical pregnancy was defined by the presence, on transvaginal ultrasound, of a gestational sac and fetal cardiac activity after 6 weeks of gestation. Ongoing pregnancy was defined as ultrasound demonstration of a living fetus after 12 week of gestation. Multiple pregnancies were defined as showing more than two intrauterine fetal cardiac activities at ultrasound. Our primary outcomes were ongoing pregnancy rate and multiple pregnancies rates.

4. Statistical analysis

Statistical analysis was performed using Statistical Program for Social Science (SPSS Inc., Chicago, IL,

Table 1. Reproductive profiles of patients undergoing day 3 and day 5 embryo transfer (ET) in fresh *in vitro* fertilization (IVF) cycles

Parameter	Day 3 ET (n=90)	Day 5 ET (n=90)	*p*-value
Mean age (yr)	32.0±2.6	32.8±3.8	NS
Duration of infertility (yr)	3.5±2.2	3.8±2.5	NS
Previous IVF cycles (n)	0.5±1.1	0.7±1.0	NS
Infertility diagnosis (n)			NS
Tubal	30 (33.3)	25 (27.8)	
Male	18 (20.0)	20 (22.2)	
Endometriosis	5 (5.6)	5 (5.6)	
Unexplained	33 (36.7)	32 (35.65)	
Others*	4 (4.4)	8 (8.9)	

*Other infertility factors include peritoneal, uterine, cervical, and anovulatory factors.
Values are mean ± SD or number (%).
Day 3 ET, embryos transferred on day 3 of culture; Day 5 ET, embryos transferred on day 5 of culture, equal number of embryos (n=2) transferred in both group; NS, not statistically significant.

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USA) software. Continuous variables were reported as mean and standard deviation or as median and range, depending on their distribution, with normal distribution defined using the Kolmogorov-Smirnov test. Between-group comparisons of normally distributed variables were assessed using the Student's *t*-test. Categorical variables were compared using the chi-square test. The significance level for all analyses was set at $p<0.05$.

RESULTS

Overall, 180 patients fulfilled our retrospective study criteria (Day 3 ET group, n=90 vs. Day 5 ET group, n=90). In comparison of reproductive profiles of patients undergoing day 3 ET and day 5 ET, there were no differences between the two groups regarding age, duration of infertility, previous IVF cycles, and the cause of infertility (Table 1).

The cycle characteristics of both groups are listed in Table 2. Two groups did not show differences in the stimulation protocol, basal FSH and E_2 levels, E_2 levels on hCG administration day, stimulation days, endometrial thickness checked on hCG adminstration day, and use of ICSI.

With regard to the oocyte and embryo characteristics, there were no differences in the number of oocytes retrieved, mature oocytes fertilized, and embryos on culture day 3 in both groups (Table 2). The pregnancy outcomes of both groups are summarized in Table 3. Day 3 ET group and day 5 ET group showed comparable results: implantation rate (39.4% vs. 32.8%), positive pregnancy rate (57.8% vs. 46.7%), clinical pregnancy rate (53.3% vs. 45.6%), ongoing pregnancy rate (50.0% vs. 42.2%), miscarriage rate (13.5% vs. 9.5%),

Table 2. Cycle characteristics and oocyte and embryo characteristics of day 3 and day 5 embryo transfer (ET) in fresh fresh *in vitro* fertilization (IVF) cycles

Parameter	Day 3ET (n=90)	Day 5ET (n=90)	*p*-value
Stimulation protocols (n)			NS
GnRH antagonist	42 (46.7)	37 (41.1)	
GnRH agonist long	48 (53.3)	53 (58.9)	
Basal FSH (U/mL)	6.7±1.7	6.6±2.1	NS
Basal E_2 (pg/mL)	28.6±12.6	29.1±12.5	NS
E_2 on hCG administration day (pg/mL)	1950.5±916.9	1949.4±924.9	NS
Stimulation days	11.0±1.4	10.9±2.2	NS
Endometrial thickness* (cm)	1.1±0.2	1.1±0.2	NS
Use of ICSI	36 (40.0)	39 (43.3)	NS
No. of oocytes retrieved	15.4±6.5	16.3±7.4	NS
No. of mature oocytes fertilized (2PN)	11.3±4.5	12.0±5.2	NS
No. of embryos on culture day 3	11.1±4.3	11.6±5.0	NS

*Endometrial thickness was checked on hCG administration day by transvaginal sonogram.
Values are mean ± SD or number (%).
ICSI, intracytoplasmic sperm injection; NS, not statistically significant; E_2, estradiol; 2PN, two pronuclei.

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Table 3. Pregnancy outcomes per patient of day 3 and day 5 embryo transfer (ET)

Parameter	Day 3ET (n=90)	Day 5ET (n=90)	*p*-value
Implantation rate (%)	71/180 (39.4)	59/180 (32.8)	NS
Pregnancy rate* (%)	52/90 (57.8)	42/90 (46.7)	NS
Clinical pregnancy rate (%)	48/90 (53.3)	41/90 (45.6)	NS
Ongoing pregnancy rate (%)	45/90 (50.0)	38/90 (42.2)	NS
Miscarriage rate (%)	7/52 (13.5)	4/42 (9.5)	NS
Multiple pregnancy rate (%)	18†/48 (37.5)	14/41 (34.1)	NS

* Pregnancy was defined as positive test for serum β-hCG level (>20 mIU/mL) 12 days after ET.
† Day 5 ET group showed one triplet pregnancies including monozygotic twining.
NS, not statistically significant.

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respectively. Both groups showed high multiple PR (37.5% vs. 34.1%). There was one triplet pregnancy (including monozygotic twining) in day 5 group. Ectopic pregnancies were 3 cases in day 3 group and 1 case in day 5 group. All of ectopic pregnancies were treated by laparoscopic surgery.

DISCUSSION

The present retrospective study suggests that day 5 (blastocyst-stage) ET is not superior to day 3 (cleavage-stage) ET on pregnancy outcomes in women with favorable conditions and good quality embryos undergoing fresh IVF. Both groups showed high multiple pregnancies rates, which means the efforts need to reduce multiple pregnancies in a selected patient population.

Theoretically, the day 5 ET seems rational due to natural selection process which does not allow most of the chromosomally abnormal embryos to reach blastocyst stage, and better synchronization with uterine endometrium. Day 5 ET is, however, associated with a significantly higher chance of not performing ET[5,6] and a significantly lower probability of embryos cryopreservation as compared with day 3 ET.[2,6] Extending culture to the blastocyst stage appears to result in attrition of some embryos that otherwise would support viable pregnancies following cleavage stage transfer, and because some chromosomally abnormal human embryos can reach the blastocyst stage *in vitro*, blastocyst culture can't be use as the sole means of identifying chromosomally abnormal embryos.

Several trials have reported an increased risk of monozygotic twining with blastocyt ET,[7~9] probably relating to alterations in the zona pellucida (ZP) or embryo hatching process. Pregnancies with monozygotic twining are considered to be high risk, because miscarriages, structural congenital anomalies (such as conjoined twins, acardiac twins, and limb reductions), excess of low birth weight, growth discordance, preterm delivery, and neurologic morbidity are more common than in dizygotic twins.[14,15] Extending culture to the blastocyst may further increase the incidence of ZP damage and harden the ZP in *in vitro* culture conditions.[8]

Blastocyst ET could be associated with altered sex ratio in the infants born, with a higher frequency of males.[16] This may be related to the observation that a more rapid development has been documented in male embryos compared with female embryos in animal

species[17~19] and humans.[20] Since there is a tendency to select the faster developing and more expanded blastocyst, the sex ratio would be altered in favor of males in blastocyst ET.[21] These risks associated with blastocyst transfer should be considered when select ET day.

It should be considered that extended culture to day 5 may be associated with risks of epigenetic modifications in offspring after ART[10,11] and at the same time, day 5 ET is associated with an increased workload, cost, and resources.[12] Accordingly physicians should consider many factors to select day 3 ET or day 5 ET, such as the number and quality of embryos, the strengths and weaknesses of each procedure, patients' preference and economic status, and the ability of laboratory.

The results of both randomized controlled clinical trials and retrospective studies examining the outcomes between day 3 ET and day 5 ET are variable. In trials with unselected populations, these studies have shown no difference in the pregnancy outcomes between day 3 and day 5 ET groups.[22~25] In a meta-analysis of 16 trials involving 1068 cycles day 2 or 3 transfers and 1048 cycles day 5 to 7 transfers, no difference were observed in the clinical pregnancy rates (15 studies; odds rations [OR]: 1.05, 95% confidence intervals [CI]: 0.88~1.26) or the live birth rate (7 studies; OR: 1.03, CI: 0.74~1.44).[6] In contrast, studies with populations of good prognosis patients (based on factors such as age, and number and quality of embryos) have shown the better implantation and pregnancy outcomes in cases of day 5 ET.[1,2] Some meta-analysis suggested that the probability of live birth was significantly higher after blastocyst-stage ET as compared to cleavage-stage ET, including equal number of embryos transfer.[26,27] A recent Cochrane review from randomized controlled trials reported that there was a significant difference in pregnancy and live birth rates in favor of blastocyst transfer in good prognosis patients, and those with high numbers of 8-cell embryos on culture day 3 are most favored.[26] However, no reliable indications to identify embryos destined to develop to viable blastocysts in vitro have been established.

Even though our present study was retrospective and small in number, included patients had favorable conditions in that they had good reproductive profiles and cycle characteristics such as a sufficient numbers of embryos on culture day 3. The results showed that there were no significant differences on implantation rate and main pregnancy outcomes between day 3 ET and day 5 ET. The results suggest that day 5 ET should not be performed preferentially to good prognosis patients unless it is considered necessary such as preimplantation genetic diagnosis, because it would be associated with a higher chance of not performing ET and a lower probability of cryopreservation of surplus embryos, and the sequential media used for blastocyst culture, which allow embryos to develop to the blastocyst stage, might be compromise their implantation potential compared with blastocysts developed *in vivo*.[28]

Our study showed high multiple pregnancies rate in the day 3 and day 5 ET group (37.5% vs. 34.1%, respectively) when only two embyros were transferred in the selected condition of both groups. As multiple pregnancies have significantly high risk for both maternal and neonatal morbidity, there have been many efforts to reduce the incidence of multiples without compromising overall pregnancy outcomes. Elective single embryo transfer (eSET) was developed in an effort for this purpose. Recent studies have suggested that eSET could reduce multiple pregnancies without significantly decreasing live birth rates, with careful patient selection and the transfer of good-quality embryo especially as blastocyst-satge single embryo was transferred.[2,29,30] A recent randomized multicenter trial showed that in women less than 36 years of age who had at least two good-quality embryos, eSET plus subsequent frozen embryo replacement dramatically reduced the rate of

multiple births and was as effective as double ET in fresh IVF cycle.[31] Our results suggest that eSET should be considered in women with good favorable conditions and good quality embryos undergoing IVF.

The present study was small in number and has the inherent bias of the retrospective design, specifically in regard of patient counseling. These biases might have influenced the results of our study. These problems can be solved with prospective randomized controlled trials.

In conclusion, day 5 ET may not be beneficial and necessary in comparison with day 3 ET, and efforts, such as eSET, are needed to decrease in multiple pregnancies in women with favorable conditions and good quality embryos undergoing IVF.

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= **Abstract** =

Objective: The aim of this study was to compare day 3 embryo transfer (D3ET) with day 5 ET (D5ET) in fresh *in vitro* fertilization (IVF) cycle on pregnancy outcomes.

Methods: We conducted a retrospective matched case control study that included 90 women with D3ET and 90 women with D5ET from January 2007 to June 2009. Subjects were matched for reproductive profiles and IVF cycle characteristics. Two good quality embryos were transferred in both groups. Pregnancy rates (PR), implantation rate, and multiple PR were compared.

Results: Demographics, stimulation parameters and embryological data were comparable in both groups. Main pregnancy outcomes with D3ET and D5ET groups were not statistically different: implantation rate (39.4% vs. 32.8%), positive PR (57.8% vs. 46.7%), clinical PR (53.3% vs. 45.6%), ongoing PR (50.0% vs. 42.2%), respectively. Both groups showed high multiple PR (37.5% vs. 34.1).

Conclusion: D5ET may not be beneficial and necessary in comparison with D3ET on pregnancy outcomes, and elective single ET should be considered to decrease multiple pregnancies in women with favorable conditions and good quality embryos undergoing IVF.

Key Words: Embryo transfer, Cleavage stage, Blastocyst transfer, Pregnancy outcomes