Comparison of the transfer of equal numbers of blastocysts versus cleavage-stage embryos after repeated failure of in vitro fertilization cycles

Meriç Karacan · Murat Ulug · Ayse Arvas · Ziya Cebi ·
Munip Berberoglugil · Melike Batukan ·
Teksen Camlibel

Received: 8 October 2013 / Accepted: 29 November 2013 / Published online: 19 December 2013
© Springer Science+Business Media New York 2013

Abstract

Purpose To evaluate the efficacy of blastocyst transfer in women with at least two previously unsuccessful in vitro fertilization-embryo transfer (IVF-ET) attempts.

Methods Retrospective analysis of 238 couples (with previous implantation failures) had equal number (two) of cleavage-stage embryos ($n=143$) or blastocysts ($n=95$) transferred in the same IVF center.

Results The clinical pregnancy rates and live-birth rates were similar in the cleavage-stage embryo transfer group and the blastocyst group (35.6 % vs. 40 % and 32.1 % vs. 35.7 %; $p>0.05$, respectively). Miscarriage rates (9.8 % vs. 10.5 %) and multiple pregnancy rates (15.6 % vs. 23.6 %) did not differ. Although implantation rate was higher with blastocyst transfer than that with day 3 transfer, it did not reach to a statistical significance (24.7 % and 19 %, respectively, $p>0.05$).

Conclusion Blastocyst transfer in ICSI cycles does not yield a better outcome than that obtained with cleavage-stage embryos in women who had unsuccessful IVF attempts previously.

Keywords Failed IVF-ET cycle · Blastocyst transfer · ICSI · Multiple implantation failures

Capsule Blastocyst transfer in ICSI cycles does not yield a better outcome than that obtained with cleavage-stage embryos in women who had multiple implantation failures.

M. Karacan (✉) · M. Ulug · A. Arvas · Z. Cebi ·
M. Berberoglugil · M. Batukan · T. Camlibel
Division of Obstetrics and Gynecology, IVF Unit,
Ota-Jinemed Hospital, Besiktas, Istanbul, Turkey
e-mail: karacanmeric@hotmail.com

Introduction

Clinical approach in women with multiple in vitro fertilization-embryo transfer failures presents a challenge. Decreased pregnancy rate through assisted reproductive technic following an unsuccessful IVF attempt has been reported [1]. Embryological and/or endometrial factors may be responsible for such repeated failures and many strategies have been evaluated to improve results. Blastocyst transfer has been proposed as an approach after repeated IVF failures for its providing a better implantation rate in some studies [2–4] but some others did not demonstrate the beneficial effect of day 5 transfer in these patients [5, 6].

Improvement of the embryo culture systems and laboratory conditions in recent years rendered a better environment for embryo survival. Potential benefits of blastocyst transfer are a better selection of viable embryos and synchronization of embryonic stage with the endometrium in the presence of reduced uterine contractions [7, 8]. Nevertheless, blastocyst transfer may have some drawbacks such as cancellation of embryo transfer because of not having a blastocyst to transfer after 5-day culture, a lower embryo cryopreservation rate and increased laboratory work.

Following the introduction of the embryo culture until the blastocyst stage in 1994, several studies reported higher pregnancy and implantation rates with blastocyst transfer in different groups of patients [9–12], but some studies did not confirm these findings [13, 14].

Most of the studies comparing blastocyst transfer to cleavage-stage embryo transfer have included groups with the unequal numbers of embryos transferred, with more cleavage-stage embryos transferred on day 3. This may distort the interpretation of the results since the advantages of better embryo selection and more physiological endometrium disappear. Thus, comparing the same numbers of blastocysts versus

cleavage-stage embryos may eliminate this confounding factor. In a meta-analysis live-birth rate was found significantly higher after blastocyst transfer as compared to cleavage-stage embryo transfer when equal number of embryos transferred [15]. In our study, IVF cycles with two blastocysts or two cleavage-stage embryos transferred were analyzed to minimize the bias.

Since its introduction in 1992 [16], ICSI has been widely used in male infertility. Although some studies have considered improved outcome with ICSI than with conventional IVF in non-male factor infertility, the clear benefit has not been defined except avoiding total fertilization failure (TFF) [17]. We performed ICSI in all cases with previously failed IVF attempts for two reasons; first, having a higher number of male infertility cases and second, decreasing the risk of TFF.

The aim of this study was to evaluate the efficacy of blastocyst transfer among patients with at least two unsuccessful IVF attempts.

Materials and methods

Patient population

A total of 238 patients who underwent ICSI-ET cycles in a private center between November 2009 and January 2013 were retrospectively analyzed. Inclusion criteria were women ≤ 40 years with normal uterine cavity and at least two previously failed IVF attempts with fresh embryo transfers. Uterine cavity was evaluated with hysterosalpingogram and hysteroscopy in all patients. It is our policy to perform hysteroscopy in all patients who had two failed IVF attempts. We included good prognosis patients having Day 3 FSH level <10 mIU/mL and at least five antral follicles present at initial sonographic examination. Basal FSH level and antral follicle count were obtained prior to controlled ovarian hyperstimulation. Antral follicle count in blastocyst transfer was significantly higher than cleavage-stage embryo transfer group (7.8 ± 2.9 vs 6.3 ± 2.8 , $p=0.02$), but basal FSH levels did not differ (Table 1).

Pregnancy was not achieved in any of the cases in the previous attempts. The numbers of previous IVF attempts as well as the number of previous day 5 and day 3 embryo transfer attempts were similar between the two groups (Table 2). All couples had at least 1 day 3 embryo transfer and some couples had both day 3 and blastocyst transfers previously.

In the recent cycle, they had 4 cleavage-stage embryos on day 3 and, either 2 cleavage-stage embryos or 2 blastocysts transferred. Presence of morphologically normal spermatozoa in the semen was the only inclusion criterion for the men. This study did not include ICSI cycles with testicular spermatozoa, or oocyte donation cycles.

Institutional Review Board approval was obtained for the collection of the data of the couples who had undergone ICSI-ET cycles in the center.

Controlled ovarian hyperstimulation (COH)

Controlled ovarian hyperstimulation was undertaken using daily recombinant FSH (Gonal-F, Merck Serono) injections starting at the third day of the menstrual cycle and dose was adjusted according to the ovarian response. GnRH-antagonist (Cetrotide, Merck Serono) was commenced on the sixth day of the COH until at least two dominant follicles reached to 17 mm in diameter. Oocyte pick-up (OPU) was performed 35 h after the recombinant hCG (Ovitrelle 250 μ g, Merck Serono) administration through transvaginal ultrasound.

Laboratory procedures

Oocytes were cultured in G-IVF-Plus (Vitrolife, Sweden) medium supplemented with 10 % human serum albumin at 37 °C under 6 % CO₂ and 5 % O₂. Cumulus–corona complex was removed by pipetting with hyaluronidase (Type VIII, Sigma, St. Louis) 2 h after oocyte collection. Intracytoplasmic sperm injection was performed in all cycles for fertilization as described elsewhere [18]. The incubation method has not been changed and the same two embryologists performed laboratory work during the study period.

Fertilization was assessed 18–20 h after ICSI, cleavage rate was checked 48–72 h after OPU. Embryos were initially cultured in a culture medium G-1 PLUS (G5 series, Vitrolife AB, Sweden) until day 3, and then transferred to G-2 PLUS (G5 series, Vitrolife AB, Sweden). Embryo transfer for cleavage-stage group was carried out using embryos having the highest embryo grading score (embryo with 7–8 even blastomers and less than 10 % fragmentation) on day 3. Cleavage-stage embryos were graded using the scoring system of the Society for Assisted Reproductive Technology (SART).

Blastocyst transfer was performed on day 5 after oocyte collection and blastocysts were assessed by Gardner's grading system [19]. Cavitating blastocysts with well-defined inner cell mass and good trophoctoderm were transferred.

Two embryos were transferred in both groups. Day of the transfer was scheduled upon either patient's preference after counseling with the physician, or laboratory's work load. Luteal phase was supported by 50 mg progesterone in oil injections IM and continued until fetal heart beat was detected. Excess embryos were cryopreserved by using vitrification technic as described previously [20].

Pregnancy was confirmed by assessing serum β -hCG level 12 days after the embryo transfer. Pregnancy test was accepted as positive when β -hCG level was more than 30 mIU/ml. Clinical pregnancy was defined by the presence of a gestational sac with the fetal heart beat on a 7-week ultrasound. The implantation rate was considered as the percentage of fetal sacs with heart beat on the number of embryos transferred. Live-birth was confirmed by the records of the hospital or telephone interviews of the couples.

Table 1 Patient characteristics of cleavage-stage and blastocyst transfer groups

Variables	Cleavage-stage ET	Blastocyst transfer	<i>p</i> value
No. of patients	143	95	
Average age of women	35.4±4.3	34.9±5.6	NS
FSH level (day 3) (mIU/ml)	7.1±2.2	6.2±3.1	NS
Antral follicle count	6.3±2.8	7.8±2.9	0.02
Duration of infertility (yrs)	5.3±2.3	5.1±2.9	NS
Etiology of infertility			
Male factor (MF) (%)	62 (43.3)	42 (44.2)	
Female factor (FF) (%)			
Anovulation	11 (7.6)	9 (9.4)	
Tubal factor	10 (6.9)	7 (7.3)	
Endometriosis	8 (5.5)	6 (6.3)	
Unexplained	28 (19.5)	19 (20 %)	
Mixed (MF + FF)	24 (16.7)	12 (12.6)	

Values are expressed as mean ± SD

NS not significant

Statistical analysis

Statistical analysis was performed with Statistics Package for Social Sciences version 13.0 (SPSS Inc. Chicago, IL) program, and the average value was expressed as the mean ± standard deviation. Data was analyzed using the Student's *t* tests or X^2 analysis where appropriate. A *P* value of <0.05 was considered statistically significant.

Results

Among 238 cycles, cleavage-stage embryos were transferred in 143 and blastocyst-stage embryos transferred in 95 cycles. Patient characteristics are shown in Table 1. There were no differences between the cleavage-stage embryo and blastocyst transfer groups in terms of the age of women and, duration and etiology of infertility. Antral follicle count was significantly higher in blastocyst group than cleavage-stage embryo group (7.8±2.9 vs. 6.3±2.8, *p*<0.02).

Regarding the outcome of the cycles, the numbers of follicles and oocytes collected were significantly higher in blastocyst group than those of cleavage-stage group (7.4±2.4 vs. 5.9±2.6; *p*<0.02 and 11.2±4.8 vs. 6.5±3.8; *p*<0.01, respectively) (Table 3). The blastocyst formation rate was 51.3 % in the blastocyst transfer group. The embryo freezing

rates were 27.2 % (39/143) in the cleavage-stage group and 24.2 % (23/95) in the blastocyst group (*p*>0.05).

Fertilization and clinical pregnancy rates were similar in both groups (72 % vs. 79 % and 35.6 % vs. 40 % in cleavage-stage ET group and blastocyst group, respectively). Implantation rate in blastocyst group seemed higher (24.7 %) than that in cleavage stage group (19 %) but it did not reach to a clinical significance (*p*>0.05). Miscarriage rate did not differ between the two groups (10.5 % in the blastocyst group and 9.8 % in the cleavage-stage embryo transfer group). Live-birth rates were similar in both groups (32.1 % vs. 35.7 %).

Nine of 38 (23.6 %) pregnancies in blastocyst transfer group and 8 of 51 (15.6 %) pregnancies in the cleavage-stage group were multiple (twins). All twin pregnancies were nonidentical. Ectopic pregnancy rates per transfer were 2.1 % (2/95) in the blastocyst group and 2.0 % (3/143) in the cleavage-stage embryo transfer group (*p*>0.05).

Regarding the number of cryopreserved embryos in each group, 124 embryos (3.1±0.8 cleavage-stage embryos per cycle) were cryopreserved in cleavage-stage group and 57 (2.4±0.6 blastocysts per cycle) in blastocyst group. In cleavage-stage group, 25 patients had frozen embryo transfers (FET) following fresh cycle and 9 of them achieved pregnancy, yielding the cumulative pregnancy rate (CPR) of 41.9 %. In blastocyst group, 16 patients had FET and 6 achieved pregnancy, giving the CPR of 46.3 %. Statistically significant

Table 2 Characteristics of previously failed transfers in cleavage-stage ET and blastocyst transfer groups

Previous transfers	Cleavage-stage ET (<i>n</i> =143)	Blastocyst ET (<i>n</i> =95)	<i>P</i> value
No. of fresh transfers	3.1±0.6	3.3±0.7	NS
No. of fresh and frozen transfers	3.4±0.8	3.7±1.1	NS
No. of Day 3 ET attempt	2.1±0.5	2.2±0.4	NS
No. of Day 5 ET attempt	0.9±0.2	0.8±0.2	NS

Values are expressed as mean ± SD

NS not significant, ET embryo transfer

Table 3 Cycle characteristics of cleavage stage and blastocyst transfer groups

Characteristics	Cleavage-stage ET (<i>n</i> =143)	Blastocyst ET (<i>n</i> =95)	<i>P</i> value
No. of follicles >15	5.9±2.6	7.4±2.4	0.02
No. of oocytes collected	6.5±3.8	11.2±4.8	0.01
Fertilization rate %	72	79	NS
No of ETs per cycle	2	2	
Embryo freezing rate (%)	39 (27.2)	23 (24.2)	NS
No. of clinical pregnancies (%)	51 (35.6)	38 (40)	NS
Implantation rate (%)	57/286 (19)	47/190 (24.7)	NS
Miscarriage rate (%)	5 (9.8)	4 (10.5)	NS
No. of multiple pregnancies (%)	8 (15.6)	9 (23.6)	NS
No. of live births (%)	46 (32.1)	34 (35.7)	NS

Values are expressed
as mean ± SD

NS not significant

difference was not observed in CPR between the two groups ($p>0.05$).

Discussion

This retrospective study aimed at evaluating potential benefits of blastocyst transfer in couples with previous implantation failures. Although several advantages of blastocyst transfer have been presented in the previous studies, our findings are not in favor of prolonged culture on the outcome of patients with previously failed IVF attempts. In a selected population with 4 embryos available on day 3, live-birth rates were similar following both two cleavage-stage embryos transfer and two blastocysts transfer. The comparison between the two groups was retrospective, but two groups were closely matched in regard to demographic characteristics (e.g., good prognosis patients) and the number of embryos transferred was identical. Number of follicles and oocytes obtained were higher in blastocyst transfer group than those in cleavage-stage group. Thus, this group may represent a group of better responders. However, it could be accepted as a potential bias if the pregnancy rate were in favor of blastocyst transfer group.

All cycles were managed with a fixed protocol by the same physician and embryology team. A potential weakness of this study is the inherent bias of the retrospective design, particularly regarding physician's attitude in counseling for the stage of embryo transfer.

An embryo is most sensitive to its environment prior to compaction and any stress will be greatest at the cleavage stage [21]. Delaying embryo transfer after the compaction starts may reduce embryonic stress. Human gene expression first occurs between the 4 and 8-cell stages and only embryos achieving transition from maternal to embryonic genome might continue developing to reach the blastocyst stage [22]. Thus, theoretically prolonged culture may provide a mechanism for a better selection of the healthiest embryos. Nevertheless, there exist conflicting reports regarding the benefit of

blastocyst transfer in patients undergoing IVF. These results may be due to different culture media used or laboratory conditions in various studies.

Gardner et al. reported similar pregnancy but significantly higher implantation rate with blastocyst stage transfer compared to day 3 transfer (50.5 % vs. 30.1 %, $p<0.01$) in a prospective randomized trial in good prognosis patients [23]. Subsequent studies have generally reported higher live-birth rates after blastocyst transfer than cleavage-stage embryo transfer in good prognosis patients [9–12] but some studies did not confirm these findings [13, 14]. Moreover, Balaban et al. reported higher implantation rate after blastocyst transfer than day 3 embryo transfer (15 % vs. 5.9 %, respectively) when poor quality embryos were transferred in each group [24].

In a recent meta-analysis, it was concluded that a small significant difference exists in live birth rates in favor of blastocyst transfer (Day 5 to 6) compared to cleavage stage transfer (Day 2 to 3) [25]. However, cumulative clinical pregnancy rates obtained through cleavage-stage embryo transfers (derived from fresh and thaw cycles) were found higher than those from blastocyst transfers.

Effect of blastocyst transfer on patients with previously failed IVF attempts was evaluated in many studies with incompatible results. Initial studies suggested that delayed embryo transfer might be beneficial in this group of patients but results can not be extrapolated as the benefit of blastocyst transfer because of the lack of the control group [9, 26, 27].

Simon et al. reported higher implantation and pregnancy rates in patients with previously failed IVF attempts and undergoing ovum donation with blastocyst transfer than those obtained with day 2 embryo transfer (32.7 % vs. 4.5 % and 54.5 % vs. 13.3 %, respectively). However, results were similar between blastocyst transfer and day 2 embryo transfer groups in non-donor IVF cycles (implantation rates were 11.8 % vs. 10.7 % and pregnancy rates were 20.2 % and 35 %, respectively) [28].

In a retrospective study, increased implantation and pregnancy rates with blastocyst transfer compared to day 3 embryo transfer (11.3 % vs. 3.4 % and 40 % vs. 9.1 %, respectively)

were reported in patients with multiple failed IVF cycles [2]. The number of patients in each arm was limited in this study. Additionally, a prospective non-randomized study comparing blastocyst transfer with day 2 transfer in patients who had at least two early embryo transfer cycles previously found higher implantation and clinical pregnancy rates with blastocyst transfer (25.4 % vs. 12.4 % and 34.1 % vs. 22.4 %, $p < 0.05$, respectively) [3]. Although live-birth rate per cycle seemed better with blastocyst transfer (27.9 % vs. 19.7), it did not achieve a statistical significance.

Barrenetxea et al. observed the pregnancy rate of 38 % in patients with multiple failed IVF attempts and underwent day 5 transfer [4]. Pregnancy rate was triple as high in day 5 transfer patients as day 6 transfer patients, and there was no control group having day 3 transfer in their study.

In another study, the benefit of day 5 transfer in patients with implantation failure through day 2–3 embryo transfer cycles was evaluated in a prospective study [5]. Patients were randomly allocated either to another cleavage-stage embryo transfer or blastocyst transfer. Implantation rate was significantly higher in blastocyst transfer patients than that obtained in day 3 transfer patients (21.1 % vs. 6 %, respectively). Although blastocyst transfer yielded a better clinical pregnancy rate than day 3 transfer (21.7 % vs. 12.9 %, respectively), the difference did not reach to statistical significance. The live-birth rates per retrieval were similar between the two groups (10.3 % cleavage stage vs. 13 % blastocyst stage). In a retrospective study, Shapiro et al. reported a dramatic decline in implantation and pregnancy rates in patients undergoing repeated blastocyst transfer cycles even after one failed IVF attempt [6]. Our findings are in accordance with the later studies that did not observe a better outcome with blastocyst transfer in patients with previous implantation failure. Hypothetically, culturing embryos until blastocyst stage provides an opportunity to select better/viable embryos. It would be a reliable approach to culture 5 days and transfer blastocysts in patients who had previous implantation failure. However, we did not detect the superiority of this approach over cleavage-stage embryo transfer in these patients. Thus, despite reaching the blastocyst stage, not all blastocysts can result in healthy pregnancies, assumingly due to some intrinsic factors expressed after blastocyst stage and/or endometrial factors being responsible for consecutive implantation failures.

ICSI has been commonly used in in vitro fertilization procedures for couples with male factor infertility. Since impaired semen quality may have a negative impact on the development of embryos, the influence of ICSI on blastocyst development is still a matter of debate. Some studies reported decreased blastocyst development [14, 29] in ICSI than IVF embryos whereas others did not observe any difference [22, 30]. Rienzi et al. reported blastocyst formation rate as 44.8 % in ICSI cycles [30]. The blastocyst formation rate (51.3 %) observed in the present study was comparable to those

reported with conventional IVF (41 % reported by Huisman G. et al., 38 % reported by Karaki RZ. et al.) [31, 32]. Even though ICSI did not seem detrimental this result can not be extrapolated that blastocyst development is not affected by poor sperm quality as we employed ICSI in all patients including male and non-male factor infertility.

Blastocyst transfer has been offered to reduce the risk of multiple pregnancy rate by selecting only one or two embryos with the best chance of implantation [14, 33, 34]. When replacing two embryos, the twinning rates in the present study were 23.6 % after blastocyst transfer and 15.6 % after day 3 embryo transfer. Thus, in a patient with multiple implantation failure transfer of two blastocysts does not seem to be a reliable approach to lower the risk of twin pregnancies.

It has been postulated that the number of supernumerary embryos for freezing be reduced in day 5 transfer cycles [2, 35]. This may decrease the cumulative pregnancy rate following consecutive blastocyst transfers. In our study, the percentage of cycles achieving embryo cryopreservation were similar between day 3 embryo transfer group and blastocyst transfer group (27.2 % vs. 24.2 %). Development of vitrification technic enabled physicians to freeze higher number of blastocysts as well as reducing the cost of the storage of the embryos [36]. Cumulative pregnancy rates were similar between cleavage-stage group (41.9 %) and in blastocyst transfer group (46.3 %) after all embryos were transferred in our study.

It has been assumed that the risk of ectopic pregnancy could be decreased following day 5 transfer compared to day 3 embryo transfer because of the reduced uterine contractility and the larger diameter of the blastocyst [8, 37]. In the present study, the ectopic pregnancy rates were identical (2.1 % in the blastocyst group and 2.0 % in the cleavage-stage embryo transfer group). Although higher pregnancy loss was reported with day 3 embryo transfer than day 5 embryo transfer [38], recent Cochrane meta-analysis including 13 studies did not find any difference [24]. Miscarriage rates were similar in our study (9.8 % vs. 10.5 %), which is in accordance with the recent meta-analysis. A possible increase of monozygotic twinning related to blastocyst transfer has been reported [39]. However, we observed no monozygotic twinning neither in cleavage-stage embryo transfer nor in blastocyst transfer patients.

Conclusion

Blastocyst transfer in ICSI cycles does not yield a better outcome than that obtained with cleavage-stage embryos in women who had unsuccessful IVF attempts previously. Further prospective randomized studies are needed to confirm this finding.

Acknowledgments We thank Romina Pakertik and Yıldız Mutluer for collecting the data and statistical analysis.

References

- Templeton A, Morris JK, Parslow W. Factors that affect outcome of in vitro fertilization treatment. *Lancet*. 1996;348:1402–6.
- Cruz JR, Dubey AK, Patel J, Peak D, Hartog B, Gindoff PR. Is blastocyst transfer useful as an alternative treatment for patients with multiple in vitro fertilization failures? *Fertil Steril*. 1999;72:218–20.
- Guerif F, Bidault R, Gasnier ML, Couet ML, Gervereau O, Lansac J, et al. Efficacy of blastocyst transfer after implantation failure. *Reprod Biol Online*. 2004;9:630–6.
- Barrenetxea G, Lopez de Larrueza A, Ganzabal T, Jimenez R, Carbonero K, Mandiola M. Blastocyst culture after repeated failure of cleavage-stage embryo transfers: a comparison of day 5 and day 6 transfers. *Fertil Steril*. 2005;83:49–53.
- Levitas E, Lunenfeld E, Har-Vardi I, Albotiano S, Sonin Y, Hackmon-Ram R, et al. Blastocyst-stage embryo transfer in patients who failed to conceive in three or more day 2-3 embryo transfer cycles: a prospective, randomized study. *Fertil Steril*. 2004;81:567–71.
- Shapiro BS, Richter KS, Harris DC, Daneshmand ST. Dramatic declines in implantation and pregnancy rates in patients who undergo repeated cycles of in vitro fertilization with blastocyst transfer after one or more failed attempts. *Fertil Steril*. 2001;76:538–42.
- Gardner DK, Balaban B. Choosing between day 3 and day 5 embryo transfer. *Clin Obstet Gynecol*. 2006;49:85–92.
- Fanchin R, Ayoubi JM, Righini C, et al. Uterine contractility decreases at the time of blastocyst transfers. *Hum Reprod*. 2001;16:1115–9.
- Olivennes F, Hazout A, Lelaidier C, et al. Four indications for embryo transfer at the blastocyst stage. *Hum Reprod*. 1994;9:2367–73.
- Wilson M, Hartke K, Kiehl M, Rodgers J, Brabec C, Lyles R. Integration of blastocyst transfer for all patients. *Fertil Steril*. 2002;77:693–6.
- Milki AA, Hinckley MD, Fisch JD, Dasig D, Behr B. Comparison of blastocyst transfer with day 3 embryo transfer in similar patient populations. *Fertil Steril*. 2000;73:126–9.
- Papanikolaou EG, D'haeseleer E, Verheyen G, Van de Velde H, Camus M, Steirteghem A, et al. Live birth rate is significantly higher after blastocyst transfer than after cleavage-stage embryo transfer when at least four embryos are available on day 3 of embryo culture. *Hum Reprod*. 2005;20:3198–203.
- Utsunomiya T, Ito H, Nagaki M, Sato J. A prospective randomized study: day 3 versus hatching blastocyst stage. *Hum Reprod*. 2004;19:1598–603.
- Bungum M, Bungum L, Humaidan P, Andersen CY. Day 3 versus day 5 embryo transfer: a prospective randomized study. *Reprod Biomed Online*. 2003;7:98–104.
- Papanikolaou EG, Kolibianakis EM, Tournaye H, Venetis CA, Fatemi H, Tarlatzis B, et al. Live-birth rates after transfer of equal numbers of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis. *Hum Reprod*. 2008;23:91–9.
- Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*. 1992;4:17–8.
- Ola B, Afnan M, Sharif K, et al. Should ICSI be the treatment of choice for all cases of in-vitro conception? Considerations of fertilization and embryo development, cost-effectiveness and safety. *Hum Reprod*. 2001;16:2485–90.
- Van Steirteghem AC, Nagy P, Joris H, Liu J, Staessen C, Smits J, et al. High fertilization rates after intracytoplasmic sperm injection. *Hum Reprod*. 1993;8:1061–6.
- Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. *Toward Reproductive Certainty: Fertility and Genetics Beyond*, vol. 199. London: Parthenon Publishing; 1999. p. 378–88.
- Isachenko V, Katkov II, Yakovenko S, Lulat AG, Ulug M, Arvas A, et al. Vitrification of human laser treated blastocysts within cut standard straws (CSS): novel aseptic packaging and reduced concentrations of cryoprotectants. *Cryobiology*. 2007;54(3):305–9.
- Gardner DK, Lane M. Ex vivo early embryo development and effects on gene expression and imprinting. *Reprod Fertil Dev*. 2005;17:361–70.
- Braude P, Bolton V, Moore S. Human gene expression first occurs between the four and eight cell stages of preimplantation development. *Nature*. 1988;332:459–61.
- Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. *Hum Reprod*. 1998;13:3434–40.
- Balaban B, Urman B, Alatas C, Mercan R, Aksoy S, Isiklar A. Blastocyst-stage transfer of poor-quality cleavage-stage embryos results in higher implantation rates. *Fertil Steril*. 2001;75(3):514–8.
- Glujovsky D, Blake D, Farquhar C, Bardach A. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev*. 2012 Jul 11;7:CD002118.
- Jayot S, Pameix I, Verdaguer S, et al. Coculture of embryos on homologous endometrial cells in patients with repeated failures of implantation. *Fertil Steril*. 1995;63:109–14.
- Guerin JF, Nicollet B. Interest of co-culture for embryos obtained by in vitro fertilization. A French collaborative study. *Hum Reprod*. 1997;12:1043–6.
- Simon C, Mercader A, Garcia-Velasco J, Nikas G, Moreno C, Remohi J, et al. Coculture of human embryos with autologous human endometrial epithelial cells in patients with implantation failure. *J Clin Endocrinol Metab*. 1999;84:2638–46.
- Miller JE, Smith TT. The effect of intracytoplasmic sperm injection and semen parameters on blastocyst development in vitro. *Hum Reprod*. 2001;16:918–24.
- Karaki RZ, Samarraie SS, Younis NA, et al. Blastocyst culture and transfer: a step toward improved in vitro fertilization outcome. *Fertil Steril*. 2002;77:114–8.
- Rienzi L, Ubaldi F, Iacobelli M, Ferrero S, Minasi MG, Martinez F, et al. Day 3 embryo transfer with combined evaluation at the pronuclear and cleavage stages compares favourably with day 5 blastocyst transfer. *Hum Reprod*. 2002;17(7):1852–5.
- Huisman GJ, Fauser BC, Eijkemans MJ, Pieters MH. Implantation rates after in vitro fertilization and transfer of a maximum of two embryos that have undergone three to five days of culture. *Fertil Steril*. 2000;73:117–22.
- Racowsky C, Combelles CMH, Nureddin A, Pan Y, Finn A, Miles L, et al. Day 3 and day 5 morphological predictors of embryo viability. *Reprod Biomed Online*. 2003;6(3):323–31.
- Gardner DK, Vella P, Lane M, Wagley L, Schlenker T, Schoolcraft WB. Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. *Fertil Steril*. 1998;69(1):84–8.
- Behr B, Gebhardt J, Lyon J, Milki AA. Factors relating cryopreserved blastocyst transfer program. *Fertil Steril*. 2002;77:697–9.
- Van den Abbeel E, Camus M, Verheyen G, Van Waesberghe I, Devroey P, Van Steirteghem A. Slow controlled-rate freezing of sequentially cultured human blastocysts: an evaluation of two freezing strategies. *Hum Reprod*. 2005;20:2939–45.
- Schoolcraft WB, Surrey ES, Gardner DK. Embryo transfer techniques and variables affecting success. *Fertil Steril*. 2001;76:863–70.
- Papanikolaou EG, Camus M, Fatemi HM, et al. Early pregnancy loss is significantly higher after day 3 single embryo transfer than after day 5 single blastocyst transfer in GnRH antagonist stimulated IVF cycles. *Reprod Biomed Online*. 2006;12:60–5.
- Shiner E, Har-Vardi I, Potashnik G. The potential association between blastocyst transfer and monozygotic twinning. *Fertil Steril*. 2001;75:217–8.