Top quality embryos at day 2: a prerequisite for single blastocyst transfer? An observational cohort study in women under 36

Fabrice Guerif · Malak Lemseffer · Milène Blanchard · Dominique Royere

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Abstract

Purpose While extended culture has been considerably improved, some questions remain regarding the application of Single Blastocyst Transfer (SBT).

Methods An observational cohort study was undertaken with 456 women under 36 years old and assigned to SBT on a voluntary basis. The main outcome was the cumulative delivery rate per couple according to the number of Top Quality Embryos (TQE) on day 2 (Group 1= ≥ 2 TQE, Group 2= 1 TQE and Group 3= 0 TQE).

Results Rate of transfer and mean number of frozen blastocysts were higher in Group 1 compared to Group 3. As a consequence, the cumulative delivery rate per couple was higher in Group 1 (47.9%) compared to Group 3 (34.9%).

Conclusions Single blastocyst transfer combining fresh and frozen cycles, might be a worthwhile strategy irrespective of embryo quality on day 2 providing good delivery rates while keeping the rate of multiple deliveries low.

Capsule Couples with ≥ 2 TQE have increased rate of cumulative deliveries after single blastocyst transfer following fresh and frozen cycles compared to couples without TQE.

F. Guerif (✉) · M. Lemseffer · M. Blanchard · D. Royere
Service de Médecine et Biologie de la Reproduction,
CHRU Bretonneau,
37000 Tours, France
e-mail: guerif@med.univ-tours.fr

F. Guerif · M. Lemseffer · M. Blanchard · D. Royere
Université François Rabelais,
37000 Tours, France

F. Guerif · M. Lemseffer · M. Blanchard · D. Royere
INRA UMR 85 - CNRS UMR 6175,
37380 Nouzilly, France

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Introduction

The goal of all assisted reproductive technology (ART) programmes is to reduce the number of embryos transferred to minimize multiple pregnancies without compromising the patient's chance of achieving a delivery [1]. Single embryo transfer has been advocated as a strategy to reduce the frequency of multiple births after in vitro fertilization (IVF) [2]. Recent advances in human IVF have been the result of the development of sequential media designed to promote embryo growth through day 5/6 of development. The use of sequential media has demonstrated that extended embryo culture to the blastocyst stage enhances the likelihood of pregnancy [3–5]. Some studies have recently reported high pregnancy rates after single blastocyst transfer (SBT) on day 5 in selected couples [6–8].

The main advantage of transferring the embryo at the blastocyst stage rather than at the cleavage stage is that extending the embryo culture allows the selection of embryos with ongoing developmental potential. Indeed, embryo selection based on morphologic criteria had certain limitation, resulting in the transfer of embryos that are chromosomally abnormal [9, 10] or that arrest at later developmental stages [11–13]. On the other hand, a potential downside to the use of extended embryo culture is that some embryos are not able to reach the blastocyst stage in culture, leading to an increase in cancelled transfers [14, 15]. It has therefore been suggested that there is a need

to develop a policy that would be able to identify patients who would benefit from the extension of culture to day 5/6, without reducing their chances of having a blastocyst transfer. According to such a view, patients with at least three [16, 17] or four [18] morphologically good embryos on day 3 should be offered a blastocyst embryo transfer. These reports were based on selected populations of patients who were at greater risk of high-order multiple gestations. It was shown in a previous study that transfer of blastocysts in patients having only fair or poor quality embryos on day 3 was feasible and associated with higher implantation rates compared to transfer of similar quality embryos on day 3 [19]. However, in all these studies the mean number of blastocysts transferred was more than two. To the best of our knowledge, the efficacy of single blastocyst transfer irrespective of embryo quality on day 2 and combining fresh and frozen transfers was never assessed.

Women under 36 and performing a first or a second IVF attempt have high risk of multiple pregnancies. However, is it reasonable to propose single blastocyst transfer to such couples without TQE on day 2? In order to answer this question, the main outcome of the present observational cohort study was the cumulative delivery rate in women under 36 after single blastocyst transfer in relation to the number of top quality embryos (TQE) available on day 2, including both fresh and frozen cycles.

Materials and methods

An observational cohort study was undertaken at the IVF unit, Bretonneau University Hospital, Tours, France, between January 2003 and July 2007. During the study period, women under 36 ($n=2687$) in the course of the first or second IVF attempt were clearly informed by a physician about the risks of multiple pregnancies. During follow-up appointments, couples were then proposed to transfer one blastocyst regardless of the number or quality of the embryos available on day 2. The theoretical drawbacks (uncertainty of reaching the blastocyst stage) and advantages (embryo selection after genome activation, more accurate synchrony between blastocyst and endometrium, and lower uterine contraction at the time of blastocyst transfer) of extended culture were explained. The choice of SBT strategy was therefore set on a complete voluntary basis.

The volunteers ($n=456$) were significantly younger compared to the non-volunteers ($n=2231$): [Female age: 30.8 ± 3.7 (volunteers) vs. 32.9 ± 4.3 years (non-volunteers), $p < 0.0001$ and male age: 34.0 ± 5.2 (volunteers) vs. 35.3 ± 5.7 years (non-volunteers), $p < 0.01$]. However, regarding biological and clinical characteristics (rate of normal

fallopian tubes, ovarian reserve, endometriosis rate, sperm concentration, sperm motility, normal spermatozoa and duration of infertility), volunteers did not differ from non-volunteers.

The inclusion criteria were the following: (a) women under 36, (b) couples performing a first or second IVF attempt, (c) couples scheduled on a voluntary basis for single blastocyst transfer (d) each volunteer couple was included once in the study.

In our study, volunteer couples were allocated to three groups according to number of TQE available on day 2 (Group 1=at least 2 TQE, Group 2=only one TQE and Group 3=no TQE).

The ovarian stimulation protocol and the IVF procedures used [classical IVF or intracytoplasmic sperm injection (ICSI)] have already been described elsewhere [20]. Endometrial thickness was measured by the same sonographer on the day of human Chorionic Gonadotrophin (hCG) administration. It was defined as the maximal distance between the echogenic interfaces of the myometrium and the endometrium and was measured in the midsagittal plane by two dimensional transvaginal ultrasound. Embryo culture with sequential media (until day 2 or day 5/6) and assessments were carried out as previously described [13]. Briefly, fertilization (day 0) was performed in G-Fert™ (Vitrolife, Gothenburg, Sweden). The following morning (day 1), the oocytes were individually placed in microdrops (25 μ l) in G1™ (Vitrolife, Gothenburg, Sweden) under mineral oil. From day 3 to day 5/6, embryo culture was performed in microdrops (25 μ l) in G2™ (Vitrolife, Gothenburg, Sweden) under mineral oil. All cultures were performed in incubators at 37°C with 6% CO₂, 5% O₂ and 89% N₂.

All subsequent optical assessments were performed using an inverted microscope with Hoffman modulation contrast (X200 and X400 magnification). Oocytes were checked for fertilization 18–20 h after insemination/ICSI (day 1). On day 2, individually cultured embryos were evaluated 44–46 h post-insemination/ICSI on the basis of the number of blastomeres, blastomere size, fragmentation rate and presence of multinucleated blastomeres. Embryos with one or more multinucleated blastomeres were excluded from further extended embryo culture. Only embryos with four similar blastomeres, less than 20% fragmentation and no multinucleated blastomeres were classified as Top Quality Embryos (TQE).

Both the outcome of extended embryo culture and the day of blastocyst development (day 5/6) were recorded for each individually cultured embryo. Blastocyst assessment was based on the expansion of the blastocoele cavity and the number and cohesiveness of the inner cell mass and trophectoderm cells [21]. A good quality blastocyst was defined as having on day 5, a well expanded blastocoele cavity (B3–B5 stages), a well defined inner cell mass [many

cells tightly packed (type A) or several cells loosely grouped (type B)] and multicellular trophectoderm [many cells forming a cohesive epithelium (type A) or few cells forming a loose epithelium (type B)]. On day 5, a full (B3) or an advanced (B4–B5) blastocyst with a good quality inner cell mass (type A or B) and multicellular trophectoderm (type A or B) was preferably selected for transfer. If none of the embryos fulfilled these criteria, one early blastocyst (B1–B2) was transferred. When no blastocyst was available on day 5, the transfer was postponed until day 6. On day 6, blastocyst transfer was carried out according to the same criteria described above. When no embryo was able to reach at least the early blastocyst-stage (B1) the transfer was cancelled. Only blastocysts at the B2–B5 stages on day 5 or at the B3–B5 stages on day 6 with A/B inner cell mass and A/B trophectoderm were frozen according to procedures of cryopreservation that have been described previously [22]. When blastocyst cryopreservation was possible, only one blastocyst was frozen per straw. For all couples, a single blastocyst was transferred from cryopreserved cycles when a transfer was available after thawing.

Serum hCG levels were measured 7 days after blastocyst transfer. Clinical pregnancy was defined as the presence of a gestational sac with foetal heart activity on ultrasound examination 5 weeks after oocyte retrieval. The implantation rate was defined as the number of gestational sacs divided by the number of blastocysts transferred.

Statistical analysis

Statistical analysis was performed using Statview 4.1 software (Abacus Concepts, Berkeley, CA, USA). Quantitative variables were compared using variance analysis followed by Fisher's PLSD test, and qualitative data were compared using contingency tables (Chi 2 test). Differences were considered significant if $p < 0.05$.

Results

A total of 456 infertile couples were assigned to the transfer of a single blastocyst for the first or second IVF attempt at the IVF unit of the University Hospital of Tours. Each couple was included once in the study. Couples were allocated to three groups according to the number of TQE available on day 2 [Group 1=at least 2 TQE (165 cycles); Group 2=only one TQE (125 cycles); Group 3=no TQE (166 cycles)].

There was no difference between groups in terms of patient characteristics (Table 1). The total number of oocytes retrieved, number of mature oocytes and fertilization rates did not differ between groups (Table 2). However, the number of embryos available on day 2 was significantly

higher in Group 1 compared to Group 2 and 3 [6.8 ± 3.4 (Group 1), 6.0 ± 3.0 (Group 2), 5.8 ± 3.0 (Group 3), $p < 0.05$]. As a consequence, the mean number of embryos allocated to extended culture until day 5/6 increased with the number of TQE available on day 2. Blastocyst development rate, percentage of cycles with blastocyst cryopreservation and mean number of frozen blastocysts were the highest when at least two TQE were available on day 2 ($p < 0.05$).

The clinical outcome of the three groups is shown in Table 3. The rate of blastocyst transfer was significantly lower when no TQE were available on day 2 [88% (Group 3), 98% (Group 2), 98% (Group 1), $p < 0.0001$]. The frequency of good quality blastocysts transferred decreased significantly with the number of TQE available on day 2 (Group 1: 79.0%, Group 2: 61.8% and Group 3: 45.2%, $p < 0.0001$). As a consequence, delivery rate per oocyte retrieval was significantly lower in Group 3 compared to Group 1 [32.5% (Group 3), 43.6% (Group 1)]. The overall miscarriage rate was 10.5% while the rate of ectopic pregnancies was 1.6%. The clinical implantation rate ranged from 39.0% (Group 2) to 51.9% (Group 1). The overall multiple delivery rate (only monozygotic twins) was 2.4%. Regarding to blastocyst quality whatever the group, we observed that the implantation rate was significantly higher when a good quality blastocyst was transferred compared to other quality blastocyst: 49.6% vs. 35.6%, respectively, $p < 0.01$. However, when a good quality blastocyst was transferred, the implantation rate of such blastocyst was similar between Group 1 and Group 3 (53% vs. 49%, respectively). Similarly, when a no-good quality blastocyst was transferred, the implantation rate of such blastocyst was similar between Group 1 and Group 3 (41% vs. 38%, respectively).

For unsuccessful couples due to transfer cancellation or after fresh transfers without delivery, the cryopreservation rate was 72% in Group 1 (67/93), 53% in Group 2 (44/83) and 30% in Group 3 (34/112) ($p < 0.0001$). The percentage of couples then allocated to a thawing cycle was similar between groups [88% in Group 1 (59/67), 86% in Group 2 (38/44) and 91% in Group 3 (31/34)]. In fact some unsuccessful couples [12% in Group 1 (8/67), 14% in Group 2 (6/44) and 9% in Group 3 (3/34)] did not thaw their cryopreserved blastocysts for different reasons (loss to follow-up, divorce, move...). The biological and clinical outcome of frozen/thawed cycles is shown in Table 4. The blastocyst survival rate was similar in all groups (66% in Group 1, 69% in Group 2, and 67% in Group 3). The rate of transfer after thawing ranged from 73% (Group 2) to 82% (Group 1). When a transfer was available after thawing, only one blastocyst was transferred for each woman in all groups. The delivery rate per thawed cycle was 9.1% in Group 1, 11.5% in Group 2 and 8.7% in Group 3, without twin delivery for all groups.

Table 1 Patient characteristics in groups classified according to number of TQE available on day 2

	Group 1 ≥ 2 TQE (day 2)	Group 2 1 TQE (day 2)	Group 3 0 TQE (day 2)
Number of couples	165	125	166
Female age (years)	30.8±3.6	30.5±3.9	31.2±3.7
Range (years)	21–35	21–35	22–35
Normal Fallopian tubes	79%	79%	77%
Mean FSH (UI/L)	6.8±2.2	7.0±2.4	6.9±2.3
Endometriosis (stages III/IV)	9%	11%	11%
Male age (years)	33.4±5.5	34.5±5.4	34.2±4.7
Sperm concentration (M/ml)	36.9±44.7	41.3±49.9	36.0±39.7
Sperm motility (a+b) (%)	18±13	18±12	19±13
Normal spermatozoa (%)	8±9	6±7	8±10
Primary infertility	76%	80%	77%
Duration of infertility (years)	4.7±2.5	4.4±2.8	4.3±2.5
Endometrial thickness hCG day (mm)	9.6±2.0	9.4±1.9	9.5±1.9

Values are means ± SD

TQE top quality embryo

The cumulative number of deliveries including both fresh and frozen blastocyst transfers are summarized in Table 5. Frozen blastocyst transfers resulted in additional deliveries (7, 6 and 4 in Groups 1, 2 and 3, respectively), thus providing significantly higher cumulative delivery rate in Group 1 (47.9%) compared to Group 3 (34.9%) ($p<0.05$).

Discussion

The present observational cohort study showed that SBT was more effective in women under 36 and with at least

two TQE available on day 2 compared to couples without TQE when deliveries issued from fresh and frozen/thawed cycles were added.

Despite encouraging reports in selected patients, single embryo transfer is not widely practised worldwide. Many patients still want to have multiple embryos transferred to increase the probability of pregnancy [23]. Extending culture to day 5/6 has been advocated to identify developmentally competent embryos for transfer or cryopreservation [4, 5]. This approach should make possible the transfer of a single blastocyst, thereby maintaining high pregnancy rates while controlling the incidence of multiple gestations [6–8]. The integration of

Table 2 Biological characteristics of IVF attempts according to number of TQE available on day 2

	Group 1 ≥2 TQE (day 2)	Group 2 1 TQE (day 2)	Group 3 0 TQE (day 2)
Number of cycles	165	125	166
Rate of ICSI attempts	68%	71%	73%
Number of IVF attempts	1.3±0.4	1.3±0.5	1.3±0.5
Number of total oocytes	11.2±5.6	10.6±5.1	10.7±5.9
Number of mature (MII) oocytes	8.7±4.5	8.3±3.9	8.0±4.1
Fertilization rate	80%	79%	78%
Number of embryos on day 2	6.8±3.4 ^{a,b}	6.0±3.0 ^a	5.8±3.0 ^b
Total number of embryos on day 2	1121 ^{a,b}	747 ^a	960 ^b
Number of embryos in extended culture	6.3±3.1 ^{a,b}	5.3±2.3 ^a	4.9±2.3 ^b
Blastocyst rate (day 5/6)	68% ^{a,b}	56% ^{a,c}	50% ^{b,c}
Number of blastocysts on day 5/6	4.2±2.6 ^{a,b}	2.8±1.5 ^a	2.5±1.8 ^b
Percentage of cycles with blastocyst cryopreservation	77% ^{a,b}	57% ^{a,c}	37% ^{b,c}
Number of frozen blastocysts	2.2±1.9 ^{a,b}	1.1±1.3 ^{a,c}	0.8±1.3 ^{b,c}

Values are means ± SD

Values with the same superscript are significantly different ($p<0.05$)

TQE top quality embryo, MII metaphase II

Table 3 Fresh cycles: Clinical outcome according to number of TQE available on day 2

	Group 1 ≥ 2 TQE (day 2)	Group 2 1 TQE (day 2)	Group 3 0 TQE (day 2)
Number of cycles	165	125	166
Number of transfers (%)	162 (98) ^a	123 (98) ^b	146 (88) ^{a,b}
Number of clinical pregnancies	82	47	62
Rate per transfer	50.6% ^a	38.2% ^a	42.5%
Rate per oocyte retrieval	49.7% ^{a,b}	37.6%	37.3% ^a
Number of gestational sacs	84	48	63
Clinical Implantation rate	51.9% ^a	39.0% ^a	43.0%
Number of miscarriages (%)	9 (11.0)	5 (10.6)	6 (9.5)
Number of ectopic pregnancies (%)	1 (1.2)	0	2 (3.2)
Number of deliveries	72	42	54
Rate per transfer	44.4%	34.1%	37.0%
Rate per oocyte retrieval	43.6% ^a	33.6%	32.5% ^a
Number of multiple deliveries (%)	2 (2.8)	1 (2.4)	1 (1.9)

Values with the same superscript are significantly different ($p < 0.05$)

TQE top quality embryo

blastocyst culture for all patients might virtually eliminate the incidence of high-order multiple pregnancies due to decrease in the number of embryos transferred [24]. Before starting infertility treatment in any given unit, patients need to be clearly informed about their chances of having a live baby according to their own situation. Information regarding delivery rates according to number of TQE available on day 2 might therefore help both physicians and patients in their decision. A previous study showed that there were differences between patients, embryologists and clinicians in their preferences in some practical aspects of blastocyst culture such as patient selection criteria [25]. Indeed, patients considered that “poor prognosis” couples were particularly suitable for blastocyst culture, whereas embryologists favoured “good prognosis” patients.

The question of the influence of embryo quality at day 2 on the efficacy of SBT strategy was addressed in this study involving 456 women under 36 and assigned

to undergo SBT for the first or second IVF attempt, irrespective of embryo quality at day 2. The aim of extending embryo culture to day 5/6 is not to improve embryo quality but to select embryos with increased probability of implantation. Our results suggest that embryo quality on day 2 is related to blastocyst development and quality. Keeping in mind that our study was based on a cohort embryo evaluation, for a defined blastocyst (good quality or not), overall embryo quality on day 2 seems helpless to predict further implantation. Moreover, the inherent difficulty with morphological scoring systems for cleavage-stage embryos is that most of them rely heavily on gross subjective observations which may not reflect the ongoing developmental potential of the embryos. When low-quality cleavage-stage embryos are developed on day 2, the choice of which to transfer is difficult. Many reports have emphasized the difficulties of correctly selecting the best embryo on day 2 [13] or on day 3 [11,

Table 4 Frozen-thawed cycles for unsuccessful couples: clinical outcome according to number of TQE available on day 2

	Group 1 ≥ 2 TQE (day 2)	Group 2 1 TQE (day 2)	Group 3 0 TQE (day 2)
Unsuccessful couples with freezing			
- Number of couples	67	44	34
Unsuccessful couples with freezing and thawing			
- Number of couples (%)	59 (88)	38 (86)	31 (91)
- Number of thawed cycles	77	52	46
Number of transfer cycles (%)	63 (82)	38 (73)	36 (78)
Blastocyst survival rate	66%	69%	67%
Mean number of transferred blastocysts	1.0±0.0	1.0±0.0	1.0±0.0
Clinical implantation rate	15.9%	18.4%	13.9%
Unsuccessful couples with freezing and thawing			
- Number of deliveries	7	6	4
- Rate per thawed cycle	9.1%	11.5%	8.7%

Clinical implantation rate =
Number of gestational sacs/
Number of transferred embryos
Delivery rate per thawed cycle =
Number of deliveries/Number of
thawed cycles

Table 5 Cumulative deliveries including fresh and frozen-thawed cycles according to number of TQE available on day 2

		Group 1 ≥ 2 TQE (day 2)	Group 2 1 TQE (day 2)	Group 3 0 TQE (day 2)
Number of couples		165	125	166
Number of deliveries	Fresh blastocyst transfer	72	42	54
	Frozen-thawed blastocyst transfer	7	6	4
	Overall results	79	48	58
	Rate per couple	47.9% ^a	38.4%	34.9% ^a
	Overall twin	2	1	1
	Rate per couple	2.5%	2.1%	1.7%

Values with the same superscript are significantly different ($p < 0.05$)

TQE top quality embryo

26]. These findings highlight the value of extended embryo culture when no TQE are available on day 2/3. It was possible to identify a group with poor quality cleavage-stage embryos (no TQE available on day 2). Such patients should be advised of the risk of their embryos not reaching the blastocyst stage. In studies including unselected patients, the cancellation rate of blastocyst transfer reported ranged from 3% [23] to 27% [27], whereas in selected patients it ranged from 4.5% [28] to 11% [29]. Only two studies reported cancellation rates of blastocyst transfer in patients without TQE on day 3 [21% [17] and 37% [30]]. By contrast, in our study, only 12% transfers were cancelled when no TQE were available on day 2. Embryo culture until the blastocyst stage might thus be proposed for couples with no TQE available on day 2 in order to perform a transfer only when a blastocyst is available. However, some authors argue that blastocyst culture might negatively affect the quality of embryo. Experiences from animal studies have shown that post-fertilization culture environment had a profound effect on the relative abundance of gene transcripts within the embryo and culture under suboptimal conditions could lead to perturbations in the pattern of expression [31, 32]. This question remains a matter of debate. The group without any TQE on day 2 was associated with decreased blastocyst rate and decreased rate of blastocyst cryopreservation. As a consequence, those couples were less often allocated to a thawing cycle when they failed in achieving a delivery after fresh transfers. This finding explained the fact that cumulative delivery rate was then significantly lower for couples without TQE compared to those who had at least two TQE on day 2.

Despite promising results after single blastocyst transfer when no TQE are available on day 2 (32.5% of delivery per oocyte retrieval), a prospective randomized study would be necessary to compare such strategy with the transfer of one or two non-TQE on day 2. We

must keep in mind that when no TQE are available on day 2, it would probably be difficult to convince couples to transfer only one embryo on day 2. Moreover, if the efficacy of transferring two non-TQE rather than one blastocyst remains to be evaluated, such strategy exposes to a risk of multiple pregnancies. No prospective randomized study comparing single blastocyst and embryo transfer when no TQE are available has been published to date.

Elective single embryo transfer has been shown to lead to similar live birth rates irrespective of the availability of good-quality embryos on day 2 [33]. With regard to blastocyst transfer, one study reported absence of pregnancy when a day 5 transfer was performed in 14 couples with no eight-cell embryos available on day 3 [17]. Some authors have therefore suggested that extended culture to the blastocyst stage might be an advantage only for patients with more than four fertilized oocytes [27] or three or more eight-cell embryos on day 3 [17]. By contrast, it has been shown that extending embryo culture to day 5 did not further compromise the outcome of a patient's cycle with three or fewer embryos on day 3 [23]. Another study concluded that blastocyst transfer was a viable alternative for patients who failed to produce good-quality cleavage-stage embryos [19]. However, in all these studies the mean number of blastocysts transferred was around or above two, whereas in our study only a single blastocyst was transferred in fresh and frozen/thawed cycles.

Finally, keeping in mind the limited number of cases included in our study, our results suggest that single blastocyst transfer combining fresh and frozen cycles, might be a worthwhile strategy for women under 36 and performing a first or second IVF attempt. For such couples, satisfying results were observed in terms of delivery while keeping low the rate of multiple deliveries, irrespective of embryo quality on day 2. However, these results have to be confirmed in further studies including more couples.

References

- Land JA, Evers JLH. Risks and complications in assisted reproduction techniques: report of an ESHRE consensus meeting. *Hum Reprod*. 2003;18:455–7.
- Bergh C. Single embryo transfer: a mini-review. *Hum Reprod*. 2005;20:323–7.
- Marek D, Langley M, Gardner D, Phil D, Confer N, Doody KM, et al. Introduction of blastocyst culture and transfer for all patients in an in vitro fertilization program. *Fertil Steril*. 1999;72:1035–40.
- Gardner DK, Phil D, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril*. 2000;73:1155–8.
- Gardner DK, Phil D, Surrey E, Minjarez D, Leitz A, Stevens J, et al. Single blastocyst transfer: a prospective randomized study. *Fertil Steril*. 2004;81:551–5.
- Henman M, Catt JW, Wood T, Bowman MC, De Boer KA, Jansen R. Elective transfer of single fresh blastocysts and later transfer of cryostored blastocysts reduces the twin pregnancy rate and can improve the in vitro fertilization live birth rate in younger women. *Fertil Steril*. 2005;84:1620–7.
- Nilsson S, Waldenström U, Engström AB, Hellberg D. Promising results with 306 single blastocyst transfers. *Fertil Steril*. 2005;83:1849–51.
- Papanikolaou EG, Camus M, Kolibianakis EM, Van Landuyt L, Van Steirteghem A, Devroey P. In vitro fertilization with single blastocyst-stage versus single cleavage-stage embryos. *N Engl J Med*. 2006;354:1139–46.
- Magli MC, Jones GM, Gras L, Gianaroli L, Korman I, Trounson AO. Chromosome mosaicism in day 3 aneuploid embryos that develop to morphologically normal blastocysts in vitro. *Hum Reprod*. 2000;15:1781–6.
- Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, Camus M, et al. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. *Hum Reprod*. 2004;19:2849–58.
- Rijnders PM, Jansen CA. The predictive value of day 3 embryo morphology regarding blastocyst formation, pregnancy and implantation rate after day 5 transfer following in-vitro fertilization or intracytoplasmic sperm injection. *Hum Reprod*. 1998;13:2869–73.
- Graham J, Han T, Porter R, Levy M, Stillman R, Tucker M. Day 3 morphology is a poor predictor of blastocyst quality in extended culture. *Fertil Steril*. 2000;74:495–7.
- Guerif F, Le Gouge A, Giraudeau B, Poindron J, Bidault R, Gasnier O, et al. Limited value of morphological assessment at days 1 and 2 to predict blastocyst development potential: a prospective study based on 4042 embryos. *Hum Reprod*. 2007;22:1973–81.
- Jones GM, Trounson AO, Lolatgis N, Wood C. Factors affecting the success of human blastocyst development and pregnancy following in vitro fertilization and embryo transfer. *Fertil Steril*. 1998;70:1022–9.
- Tsirigotis M. Blastocyst stage transfer: pitfalls and benefits. Too soon to abandon current practice? *Hum Reprod*. 1998;13:3285–9.
- 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.
- Racowsky C, Jackson KV, Cekleniak NA, Fox JH, Hornstein MD, Ginsburg ES. The number of eight-cell embryos is a key determinant for selecting day 3 or day 5 transfer. *Fertil Steril*. 2000;73:558–64.
- Papanikolaou EG, D'haeseleer E, Verheyen G, Van de Velde H, Camus M, Van 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. A randomized prospective study. *Hum Reprod*. 2005;20:3198–203.
- 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;75:514–8.
- Guerif F, Bidault R, Gasnier O, Couet ML, Gervereau O, Lansac J, et al. Efficacy of blastocyst transfer after implantation failure. *RBM Online*. 2004;9:630–6.
- Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. *Towards reproductive certainty: infertility and genetics beyond 1999: the plenary proceedings of the 11th world congress on in vitro fertilization and human reproductive genetics*. Pearl River: Parthenon; 1999. p. 378–88.
- Guerif F, Cadoret V, Poindron J, Lansac J, Royere D. Overnight incubation improves selection of frozen-thawed blastocysts for transfer: preliminary study using supernumerary embryos. *Theriogenology*. 2003;60:1457–66.
- Blennborn M, Nilsson S, Hillervik C, Hellberg D. The couple's decision-making in IVF: one or two embryos at transfer? *Hum Reprod*. 2005;20:1292–7.
- 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.
- Hartshorne GM, Lilford RJ. Different perspectives of patients and health care professionals on the potential benefits and risks of blastocyst culture and multiple embryo transfer. *Hum Reprod*. 2002;17:1023–30.
- Milki AA, Hinckley MD, Gebhardt J, Dasig D, Westphal LM, Behr B. Accuracy of day 3 criteria for selecting the best embryos. *Fertil Steril*. 2002;77:1191–5.
- Van der Auwera I, Debrock S, Spiessens C, Afschrift H, Bakelants E, Meuleman C, et al. A prospective randomized study: day 2 versus day 5 embryo transfer. *Hum Reprod*. 2002;17:1507–12.
- 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.
- Karaki RZ, Samarraie SS, Younis NA, Lahloub TM, Ibrahim MH. Blastocyst culture and transfer: a step toward improved in vitro fertilization outcome. *Fertil Steril*. 2002;77:114–8.
- Coskun S, Hollanders J, Al-Hassan S, Al-Sufyan H, Al-Mayman H, Jaroudi K. Day 5 versus day 3 embryo transfer: a controlled randomized trial. *Hum Reprod*. 2000;15:1947–52.
- Loneragan P, Rizos D, Guttierrez-Adan A, Fair T, Boland MP. Effect of culture environment on embryo quality and gene expression—experience from animal studies. *RBM Online*. 2003;7:657–63.
- Fernandez-Gonzalez R, Moreira P, Bilbao A, Jimenez A, Perez-Crespo M, Ramirez MA, et al. Long-term effect of in vitro culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. *PNAS USA*. 2004;101:5880–5.
- Van Montfoort AP, Fiddelers AA, Land JA, Dirksen CD, Severens J, Geraedts JP, et al. eSET irrespective of the availability of a good-quality embryo in the first cycle only is not effective in reducing overall twin pregnancy rates. *Hum Reprod*. 2007;22:1669–74.