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Should we consider day-2 and day-3 embryo morphology before day-5 transfer when blastocysts reach a similar good quality?

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KEY MESSAGE

Good-quality blastocyst transfer should be carried out irrespective of embryo quality at cleavage stage (day 2 or day 3), as it may not compromise success rates after IVF and intracytoplasmic sperm injection in a good-prognosis population.

A B S T R A C T

Clinical outcomes of 291 day-5 blastocyst transfers carried out between January 2012 and March 2016 were retrospectively compared according to their quality at day 2 and 3. Inclusion criteria were female age younger than 37 years; first or second IVF and intracytoplasmic sperm injection cycle; quality of the transferred blastocyst: blastocoele B3 or higher; inner-cell-mass A/B; trophectoderm A/B; and known implantation outcome for each transferred blastocyst. Blastocysts were classified into good-quality and poor-quality embryo groups at day 2 and 3. Implantation (38.7% versus 41.4), clinical pregnancy (40.3% versus 45.9%), miscarriage (22.2% versus 26.7%;) and live birth rates (37.4% versus 38.8%) were comparable in day 2 good

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and poor-quality embryo groups. No signficiant differences in morphology of transferred blastocysts at day 3 were found. Multivariable analysis highlighted that poor or good embryo quality at day 2 and day 3 were not predictive of the implantation of good-quality blastocysts (at day 2: adjusted odds ratio = 0.82 CI 95% 0.49 to 1.38; at day 3: adjusted odds ratio = 1.39; CI 95% 0.77 to 2.52). Good-quality blastocyst transfer should, therefore, be carried out irrespective of embryo quality at cleavage stage, as it may not compromise success rates in a good-prognosis population.

Introduction

Over the past decade, improvements in culture conditions have allowed the possibility of extended embryo culture until day 5, which has been increasingly preferred to a transfer at day 2 or day 3. In fact, blastocyst transfer is considered more physiological compared with transfers at cleavage stage, as it theoretically enables a better synchronization between the embryonic stage and the endometrial receptivity (Valbuena et al., 2001). Moreover, it is well known that not all embryos obtained at the early cleavage stage develop into blastocysts. Indeed, the morphological criteria commonly used to select embryos at cleavage stage are insufficient to predict their developmental potential, notably because, in many cases, they fail to discard chromosomally abnormal embryos (Fragouli et al., 2014).

Embryo culture generally improves the selection of good-quality embryos available for fresh transfer, cryopreservation, or both, and displaying a good implantation prognosis. Blastocyst morphology is usually evaluated according to three criteria defined in Gardner and Schoolcraft's classification (Gardner and Schoolcraft, 1999): the degree of blastocoele expansion, graded from B1 to B6; the inner cell mass (ICM); and the trophectoderm morphology, both graded from A to C (A corresponding to the best quality score). A high blastocyst quality has undeniably been related to the success rates after IVF or intracytoplasmic sperm injection (ICSI) attempts (Guerif et al., 2010; Rehman et al., 2007; Van den Abbeel et al., 2013). A longer period of culture, however, requires optimal culture conditions and an efficient blastocyst freezing programme to give patients all the benefit of embryo culture strategy. When these conditions are fulfilled, vitrified–warmed blastocyst transfers might provide equivalent live birth rates (LBR) as fresh transfers (Roy et al., 2014).

The efficiency of embryo culture strategy has already been demonstrated, especially in good-prognosis populations, when numerous good-quality embryos are available at cleavage stage (Glujovsky et al., 2012; Guerif et al., 2009). The overall (Guerif et al., 2009) and individual embryo qualities (Rehman et al., 2007) at cleavage stage, however, do not seem to influence the clinical outcomes after the transfer of a good-quality blastocyst, but data on this particular topic are still too scarce.

Therefore, the aim of the present study was to assess, in a good-prognosis population, the value of considering the individual embryo quality at day 2, day 3, or both, when equivalent good-quality blastocysts were available for a transfer at day 5.

Materials and methods

Study design

A retrospective study of prospectively collected data was conducted in the assisted reproduction technique unit of Jean Verdier University Hospital, Bondy, France, after being approved by the Local Ethics Committee on 18 November 2014.

Two hundred and ninety-one blastocyst transfers (125 fresh blastocyst transfers, 166 cryopreserved blastocyst transfers) carried out between January 2012 and March 2016 were retrospectively analysed. All patients met the following inclusion criteria: female age less than 37 years; first or second IVF–ICSI cycle; quality of the transferred blastocyst: blastocoele B3 or higher, inner cell mass A/B, trophectoderm A/B (according to Gardner and Schoolcraft's criteria) (Gardner and Schoolcraft, 1999); known implantation outcome for each transferred blastocyst (including double blastocyst transfers when implantation rate was 0 or 100%). Patients may have been included twice.

Study groups

Two groups were retrospectively created depending on whether the transferred blastocysts originated from a good-quality embryo at day 2 or day 3 (three to five adequately-sized blastomeres at day 2; six to 10 at day 3, <20% fragmentation, no multinucleation) or from a poor-quality embryo (embryos that did not meet the criteria mentioned above).

Sample size

A difference of 30% was previously reported by Silber (2014) between the implantation rate of good-quality blastocysts deriving from day-3 good-quality embryos (implantation rate = 70%) and day-3 poor-quality embryo (implantation rate = 40%). Therefore, considering an alpha risk less than 0.05 and a power greater than 0.95, we assumed that the sample size required to conclude was at least 69 observations in each group.

IVF procedures

Semen preparation

Semen was prepared, using a two-layer density technique (45% and 90%) of PureSperm (Nidacon International, Göteborg, Sweden) diluted in Ferticult HEPES culture media (FertiPro NV, Beernem, Belgium). After a 20-min centrifugation at 300 g, semen pellet was washed using Ferticult HEPES media (FertiPro N.V) and then centrifuged for 5 min at 600 g.

Ovarian stimulation and oocyte retrieval

Ovarian stimulation was conducted as previously described with standard agonist or antagonist protocols (Huirne et al., 2006; Sifer et al., 2006). In brief, within the 3 months preceding ovarian stimulation, antral follicle counting and serum oestradiol, FSH, and anti-Müllerian (AMH) levels were measured on cycle day 3. During a subsequent cycle, recombinant FSH therapy (Gonal-F, Serono Pharmaceuticals, Boulogne, France) was started at a dosage of 150–300 IU/day for at least 5 days, and continued until the day of HCG (Gonadotrophine Chorionique 'Endo', Organon Pharmaceuticals, Saint-Denis, France, 10,000 IU, IM) administration. From the sixth day of exogenous FSH therapy onwards,

daily FSH doses were adjusted according to oestradiol levels, the number of growing follicles, or both. During the last days of ovarian stimulation, patients had daily visits at our Institution for ultrasonographic and hormonal examinations to define the proper timing for HCG administration. Administration of HCG (day of HCG administration) was carried out as soon as three or more preovulatory follicles (16–22 mm in diameter) were observed and oestradiol levels per preovulatory follicle were greater than 200 pg/ml. Oocytes were retrieved 36 h after HCG administration by transvaginal ultrasound-guided aspiration.

Oocytes and embryo culture

Conventional IVF and ICSI procedures were carried out 3–6 h after oocyte retrieval. All cumulus oocyte complexes (COCs) were denuded before ICSI in a ready to use 80 IU/ml hyaluronidase medium (Fertipro NV, Beernem, Belgium) in accordance with the manufacturer's instructions. For an IVF attempt, COCs were inseminated with a concentration of 150,000 progressive motile sperm cells/ml. The embryos were cultured in a humidified 5% CO_2 atmosphere maintained at 37°C until day 6. The media used for embryo culture was Continuous Single Culture® (Irvine Scientific, Santa Ana, USA), from day 0 to the day of embryo transfer, and up to day 5 or day 6 if necessary. To cryopreserve potential supernumerary embryos, assessment of fertilization was carried out under an inverted microscope (x 200 magnification) 18 h after insemination (conventional IVF) or sperm injection (ICSI), by the presence of two pronuclei and two polar bodies.

Embryos were evaluated according to the conventional classification system currently used in our IVF laboratory: the number of blastomeres; the degree of cytoplasmic fragmentation; and the equality of blastomeres. Embryo morphology was scored as grade A, no cytoplasmic fragmentation; grade B, less than 20% fragmentation; grade C, 20–50% fragmentation; grade D, greater than 50% fragmentation. The number of cells per embryo was also recorded. Grade A and B embryos with three to five cells at day 2 or six to 10 at day 3, and no multinucleation were considered as good-quality embryo, respectively. Embryo quality was systematically recorded by the embryologist 44 h after insemination (day 2). Embryo culture strategy was applied to good-prognosis cases, mainly depending on the number of good-quality embryos available at cleavage stage (day 2, day 3, or both). Hence, assessment at day 3 remained optional when embryo culture strategy was already decided on day 2, thus avoiding unnecessary removals of culture dishes from optimal incubation conditions. At day 5, blastocyst morphology was evaluated according to Gardner and Schoolcraft's classification (Gardner and Schoolcraft, 1999). The blastocysts were scored according to size: B1, early blastocyst with a blastocoel less than one-half volume of that of the embryo; B2, with a blastocoel one-half volume of that of the embryo or more; B3, full blastocyst with a blastocoel completely filling the embryo; B4, expanded blastocyst with a blastocoel volume larger than that of the blastocyst, and a thinning zona; B5, hatching blastocyst with a trophectoderm starting to herniate through the zona; and B6, hatched blastocyst, having completely escaped the zona. For blastocysts graded as B3 to B6, the development of the inner cell mass and trophectoderm was assessed. The inner cell mass was graded as A, tightly packed, many cells; B, loosely grouped, several cells; C, very few cells. The trophectoderm was graded as: A, many cells forming a tightly knit epithelium; B, few cells; C, very few cells forming a loose epithelium. Good-quality blastocysts were defined as follows: blastocoel B3 or higher, inner cell mass A/B, trophectoderm A/B.

Blastocyst cryopreservation and frozen embryo cycles

In this study, only frozen embryo transfer of day-5 good-quality blastocysts were included (n = 166). Vitrification and warming procedures were carried out according to Kuwayama et al.'s method (Kuwayama et al., 2005), using embryo vitrification; freeze; and Thaw kits (Irvine Scientific, Santa Ana, USA) and high security vitrification straws HSV™ (CryoBioSystem, L'aigle, France).

Embryo transfer

Transfer of the best graded fresh or day-5 cryopreserved blastocyst(s), according to the criteria mentioned above and irrespective of their morphology at cleavage-stage, was carried out 5 days after oocyte retrieval. When several and equivalent good-quality blastocysts were available, day 2, day 3, or both, qualities were considered, explaining the higher number of cases included in the good-quality embryo groups. Luteal phase was supported with micronized progesterone (Utrogestan, Besins Iscovesco, Paris, France), 600 mg/day, administered continuously by vaginal route, starting on the evening of oocyte retrieval. Cryopreserved blastocyst transfers were scheduled during the course of either a natural, stimulated or an artificial cycle using hormone replacement treatment. In the two first cases, blastocysts were transferred on the seventh day after LH surge, and in the latter case on the fifth day after introduction of progesterone supplementation.

Clinical pregnancy was defined as presence of a gestational sac observed at ultrasound scan at around 7 weeks of amenorrhoea. All pregnancies were followed up until delivery. Miscarriages were defined as the loss of a biochemical or clinical pregnancy before 20 completed weeks of gestational age.

Statistical analysis

The primary end-point of the present investigation was implantation rate. The secondary end-points were clinical pregnancy rates (CPR) per embryo transfer, miscarriage rates and LBR.

Continuous variables are presented as median and interquartile range and qualitative variables as number and proportion. Differences between good-quality embryos and poor-qualtiy embryos were investigated by Wilcoxon–Mann–Whitney and Fisher's exact tests.

Logistic regression models were used to search for a relationship between quality of the embryo at cleavage stage (evaluated at day 2 and day 3) and other potential prognostic factors; and transfer outcomes (implantation, miscarriage and live birth). To take into account the non-independency of data that are clustered on the individual level (as a women may have several transfers), we computed clustered standard errors. Continuous variables were first entered into a univariable model using restricted cubic splines with five knots. Then, an omnibus test for the three non-linear parameters was carried out. If this test yielded a P-value less than 0.10 then the variable was entered into subsequent multivariable models using restricted cubic splines, otherwise the variable was entered as a linear term. All variables associated with transfer outcomes in univariable models ($P < 0.20$) were entered into the initial multivariable logistic regression model. A backward stepwise selection procedure with a P-value cut-off at 0.05 was then applied to identify a set of factors significantly associated with transfer outcomes (final model). Day-2 and day-3 embryo quality was forced into all multivariable models as this was the variable of interest of this study. All tests were two-sided, and $P < 0.05$ were considered statistically significant. Analyses were carried out using R statistical software, version 3.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Analysis according to the quality at day 2 of the transferred blastocyst(s)

Outcomes of 291 cycles were collected and compared according to the quality at day 2 of the transferred blastocyst(s) (D2-GQE group, *n* = 206 transfers, 238 transferred blastocysts; day 2 poor-quality embryos group, *n* = 85 transfers, 99 transferred blastocysts).

The median age of patient was 31.0 [(28.0–34.0) and 32.0 (29.0–34.0 years in day-2 good-quality embro and day-2 poor-quality embryo groups, respectively. The overall characteristics of patients, including type of infertility, ovarian stimulation and assisted reproduction techniques were similar between both groups, except for cycle rank (proportion of first IVF–ICSI cycle: 85.4% versus 72.9%; *P* = 0.02), and total dose of FSH administered (1562 [1241–2250] IU versus 1800 [1425–2550] IU, *P* = 0.03) (**Table 1**). The cycle outcomes are presented in **Table 2**. Despite comparable number of retrieved oocytes (16.0 [12.0–21.0] versus 16.0 [12.0–19.0]), more embryos were obtained in day-2 good-quality embryo group than in day-2 poor-quality embryo group (11.0 [8.0–14.0] versus 9.0 [7.0–13.0], respectively; *P* = 0.02). The proportion of single blastocyst transfers was equivalent in both groups (84.5% versus 83.5%). Interestingly, no difference in the degree of expansion (B3/4/5), quality of the inner cell mass and the trophectoderm (A/B), was noticed among blastocysts derived from day-2 good-quality embryo or poor-quality embryo. Finally, implantation rate (38.7% versys 41.4%, respectively), HCG positive rates per transfer (48.1% versus 52.9%), CPR (40.3% versus 45.9%), miscarriage rate (22.2% versus 26.7) and LBR (37.4% versus 38.8%) did no differ significantly between day-2 good-quality embryos and poor-quality embryo groups.

Table 1 – Patient characteristics and clinical parameters of the cycles according to the quality at day 2 of the transferred blastocyst [a].

	Good-quality embryos: 206 transfers; 238 transferred blastocysts	Poor-quality embryos: 85 transfers; 99 transferred blastocysts
Female age (years)	31.0 [28.0–34.0]	32.0 [29.0–34.0]
Female body mass index (kg/m^2)	24.2 [21.8–27.7]	23.5 [21.2–25.8]
Smoking, n/n (%)	34/196 (17.3)	17/82 (20.7)
Duration of infertility (years)	4.0 [3.0–5.0]	3.0 [2.0–5.0]
Primary infertility, n (%)	148 (71.8)	53 (62.4)
Indication		
Female	93 (45.1)	41 (48.2)
Male	67 (32.5)	31 (36.5)
Combined	17 (8.3)	8 (9.4)
Unexplained	29 (14.1)	5 (5.9)
IVF–ICSI first rank, n (%)[b]	176 (85.4)	62 (72.9)
Days of ovarian stimulation, n	10.0 [9.0–11.0]	10.0 [9.0–12.0]
Total FSH administered (IU)[c]	1562 [1241–2250]	1800 [1425–2550]
Progesterone day HCG (ng/ml)	0.8 [0.6–1.0]	0.8 [0.6–1.0]
Endometrium thickness (mm)	9.5 [8.1–11.4]	9.1 [7.6–11.8]

[a] Data are presented as median (interquartile range) and as number (proportion [%]).
[b] *P* = 0.02.
[c] *P* = 0.03.
ICSI, intracytoplasmic sperm injection; IU, international unit; NS, not statistically significant.

Table 2 – Biological and clinical outcomes of the cycles according to the quality at day 2 of the transferred blastocyst [a].

	Good-quality embryo: 206 transfers; 238 transferred blastocysts	Poor-quality embryo 85 transfers; 99 transferred blastocysts
Oocytes retrieved, n	16.0 [12.0–21.0]	16.0 [12.0–19.0]
Embryos obtained, n[b]	11.0 [8.0–14.0]	9.0 [7.0–13.0]
Single blastocyst transfers, n (%)	174 (84.5)	71 (83.5)
Blastocyst expansion degree, n (%)		
B3	34 (14.3)	17 (17.2)
B4	172 (72.3)	69 (69.7)
B5	32 (13.4)	13 (13.1)
Inner cell mass morphology, n (%)		
A	44 (18.5)	17 (17.2)
B	194 (81.5)	82 (82.8)
Trophectoderm morphology, n (%)		
A	39 (16.4)	17 (17.2)
B	199 (83.6)	82 (82.8)
Top blastocyst, n (%)	45 (18.9)	16 (16.2)
Implantation rate, %	38.7	41.4
HCG positive, n (%)	99 (48.1)	45 (52.9)
Clinical pregnancy rate, n (%)	83 (40.3)	39 (45.9)
Miscarriage rate, n (%)	22 (22.2)	12 (26.7)
Live birth rate, n (%)	77 (37.4)	33 (38.8)

[a] Data are presented as median (interquartile range) and as number; proportion (%).
[b] *P* = 0.02.

Analysis according to quality at day-3 of the transferred blastocyst

A similar analysis was conducted for the same cycles, when embryo quality was assessed at day 3 (day-3 good-quality embryos group, *n* = 147 transfers, 174 transferred blastocysts; day-3 poor-quality embryo group, *n* = 74 transfers, 86 transferred blastocysts). As reported in **Table 3**, patient characteristics and clinical parameters of the cycles were comparable in each group, except body mass index, which was higher in day-3 poor-quality embryo group (24.2 [22.2–27.6] kg/m^2 versus 22.9 [20.4–26.4] kg/m^2, respectively; *P* = 0.02).

Embryologic and IVF–ICSI outcomes were similar whatever the quality at day 3 of the transferred blastocyst(s) (**Table 4**). As shown, implantation rate (40.2% versus 31.4%, respectively), HCG positive rates per transfer (48.3% versus 43.2%), CPR (42.2% versus 35.1%), miscarriage rate (23.9% versus 18.8%) and LBR (36.7% versus 35.1%) were not significantly different in day-3 good-quality embryo and poor-quality embryo groups.

Table 3 – Patient characteristics and clinical parameters of the cycles according to the quality at day 3 of the transferred blastocyst[a].

	Good-quality embryo: 147 transfers; 174 transferred blastocysts	Poor-quality embryo: 74 transfers; 86 transferred blastocysts
Female age (years)	31.0 [28.0–34.0]	31.0 [29.0–34.0]
Female body mass index (kg/m²)[b]	22.9 [20.4–26.4]	24.2 [22.2–27.6]
Smoking, n/n (%)	26/139 (18.7)	11/71 (15.5)
Duration of infertility (years)	3.0 [2.0–5.0]	4.0 [2.0–5.0]
Primary infertility, n (%)	106 (72.1)	50 (67.6)
Indication		
Female	65 (44.2)	30 (40.5)
Male	53 (36.1)	28 (37.8)
Combined	15 (10.2)	4 (5.4)
Unexplained	14 (9.5)	12 (16.2)
IVF–ICSI first rank, n (%)	125 (85.0)	55 (74.3)
Says of ovarian stimulation, n	9.0 [9.0–11.0]	9.5 [9.0–11.0]
Total FSH administered (IU)	1600.0 [1243.8–2250.0]	1543.8 [1250.0–2168.8]
Progesterone day HCG (ng/ml)	0.8 [0.7–1.0]	0.8 [0.6–1.0]
Endometrium thickness (mm)	9.5 [8.1–11.5]	9.0 [7.4–11.5]

[a] Data are presented as median [interquartile range] and as number; proportion (%).
[b] $P = 0.02$.

Table 4 – Biological and clinical outcomes of the cycles according to the quality at day 3 of the transferred blastocyst.

	Good-quality embryos: 147 transfers; 174 transferred blastocysts	Poor-quality embryos: 74 transfers; 86 transferred blastocysts
Oocytes retrieved, n	16.0 [12.0–19.5]	14.0 [10.2–19.0]
Embryos obtained, n	10.0 [8.0–12.0]	9.0 [7.0–12.0]
Single blastocyst transfers, n (%)	120 (81.6)	62 (83.8)
Blastocyst expansion degree, n (%)		
B3	27 (15.5)	9 (10.5)
B4	124 (71.3)	64 (74.4)
B5	23 (13.2)	13 (15.1)
Inner cell mass morphology, n (%)		
A	36 (20.7)	16 (18.6)
B	138 (79.3)	70 (81.4)
Trophectoderm morphology, n (%)		
A	34 (19.5)	13 (15.1)
B	140 (80.5)	73 (84.9)
Top blastocyst, n (%)	37 (21.3)	19 (22.1)
Implantation rate, %	40.2	31.4
HCG positive, n (%)	71 (48.3)	32 (43.2)
Clinical pregnancy rate, n (%)	62 (42.2)	26 (35.1)
Miscarriage rate, n (%)	17 (23.9)	6 (18.8)
Live birth rate, n (%)	54 (36.7)	26 (35.1)

Data are presented as median [interquartile range] and as number; proportion (%). There were no statistically significant differences between the two groups.

Multivariable analysis

Finally, the results of multivariable analysis using implantation, HCG and live birth as clinical outcomes are presented in **Table 5** and **Table 6**. When adjusting for potential confounding factors, both embryo qualities at day 2 (**Table 5**) and day 3 (**Table 6**) were not associated with the clinical outcomes of good-quality blastocysts transfers

Table 5 – Multivariable analysis for clinical outcomes and embryo quality at day 2[a,f].

	Initial model		Final model	
	Adjusted OR	CI 95%	Adjusted OR	CI 95%
Implantation	0.86[b]	0.51 to 1.45	0.82[c]	0.49 to 1.38
HCG positive	0.87[d]	0.52 to 1.48	0.82	0.50 to 1.36
Live birth	1.00[e]	0.58 to 1.72	1.00[e]	0.58 to 1.72

[a] Multivariable logistic regression models: OR adjusted for variables when $P < 0.20$ (initial) or $P < 0.05$ (final) in the univariable analysis.
[b] Adjusted for number of blastocysts transferred, primary/secondary infertility, duration of infertility and number of days of ovarian stimulation.
[c] Adjusted for primary or secondary infertility and duration of infertility.
[d] Adjusted for number of blastocysts transferred and indication.
[e] Adjusted for number of blastocysts transferred, primary and secondary infertility and duration of infertility.
[f] All *P*-values were not statistically significant.

Table 6 – Multivariable analysis for clinical outcomes and embryo quality at day 3[e].

	Initial model		Final model	
	Adjusted OR	CI 95%	Adjusted OR	CI 95%
Implantation	1.39[a]	0.77 to 2.52	1.39[a]	0.77 to 2.52
HCG positive	1.19[b]	0.67 to 2.12	1.23	0.70 to 2.15
Live birth	1.19[c]	0.64 to 2.22	1.16[d]	0.63 to 2.15

[a] Adjusted for number of blastocysts transferred and primary and secondary infertility.
[b] Adjusted for number of blastocysts transferred and number of oocytes retrieved and primary and secondary infertility.
[c] Adjusted for number of blastocysts transferred, primary and secondary infertility, duration of infertility and IVF and intracytoplasmic sperm injectino rank.
[d] Adjusted for number of blastocysts transferred, primary and secondary infertility and duration of infertility.
[e] All *P*-values were not statistically significant.

(final model for quality at day 2 and (i) implantation: adjusted odds ratio (AOR) = 0.82 (CI 95% 0.49 to 1.38); (ii) HCG: adjusted OR = 0.82 (CI 95% 0.50 to 1.36; (iii) live birth: adjusted OR = 1.00 (CI 95% 0.58 to 1.72); final model for quality at day 3 and (i) implantation: adjusted OR = 1.39; CI 95% 0.77 to 2.52); (ii) HCG: adjusted OR = 1.23; CI 95% [0.70 to 2.15); (iii) live birth: adjusted OR = 1.16; CI 95% 0.63 to 2.15).

Discussion

The efficiency of embryo culture strategy has already been evaluated in a meta-analysis (Glujovsky et al., 2012) that compared the outcomes of transfers at day 2 and day 3 versus day 5 and day 6 reported in several prospective studies. In the case of fresh blastocyst transfer, LBR were significantly higher (12 randomized control trials, 1510 women; 31.2% at day 2 and 3 versus 38.9% at day 5 and 6; $P = 0.02$; OR 1.40 1.13 to 1.74), and especially when numerous good-quality embryos were available at day 2, day 3, or both, justifying the decision of embryo culture strategy (two RCTs, 364 women; 31.5% versus 50.0%; $P = 0.0004$; OR 2.17 1.42 to 3.33). In an observational cohort study conducted in a good-prognosis population, Guerif et al. (2009) confirmed the relevance of opting for an embryo culture on the basis of embryo quality observed at day 2. They actually reported that the number of top-quality embryos at day 2 (four even cells, <20% fragmentation rate) was predictive of the blastocyst rate (68% when more than two top-quality embryos versus 50% when no top-quality embryos was obtained at day 2; $P < 0.05$). They reported similar LBR per blastocyst transfer; however, whatever the number of top-quality embryos obtained at day 2, suggesting that once blastocysts are available, the overall embryo quality at day 2 has no affect on the subsequent clinical outcomes. Another study, published by Rehman et al. (2007), evaluated three markers of the quality and progression of 2000 embryos until blastocyst stage: the blastocyst quality score, a numerical blastocyst morphology-grading system calculated according to Gardner's criteria; the embryo progression index, calculated from the number of observed or estimated cells in the developing embryo, between day 1 and day 3; and the embryo progression index between day 1 and day 6). A receiver operator characteristic curve analysis showed that blastocyst quality score and embryo progression index day 1–6 seemed to be good predictors of clinical pregnancy, whereas embryo progression index day 1–3 seemed to be less powerful. These conclusions reinforce the fact that the better the quality of the transferred blastocyst, the higher its implantation potential.

To the best of our knowledge, no study has previously evaluated the effect of the individual embryo quality at cleavage stage on the outcome of good-quality blastocyst transfers. In fact, the present study has shown that, in a good-prognosis population, and using an appropriate statistical methodology, clinical outcomes are not influenced by good or poor quality at day 2 or day 3 when good-quality blastocysts are transferred at day 5. Even if some parameters were significantly different between good-quality embryos and poor-quality embryo at day 2 and day 3, the observed difference did not seem to negatively affect IVF–ICSI clinical outcomes. Furthermore, using multivariable logistic regression models, clinical outcomes after good-quality blastocyst transfers were not related to day 2 and day 3 embryo quality. Recently, Kaartinen et al. (2015) analysed the outcomes of 134 vitrified-warmed blastocyst transfers, using good-quality blastocysts arising from supernumerary poor-quality embryos at day 2 or day 3. A CPR of 24.6% was reported in the cryopreserved blastocyst group, whereas CPR in the group of good-quality embryos cryopreserved at day 2 or day 3 reached 29.4%. Although these results were not compared with the outcome of blastocyst transfers derived from good-quality embryos, they still suggest that poor-quality embryos at cleavage stage may develop into good-quality blastocysts, displaying good implantation potential. Moreover, Poulain et al. (2014) reported an implantation rate of 29.4% and a CPR of 30.3% after the transfer of frozen-thawed high-quality blastocysts arising from day-3 poor-quality embryos. These findings are in line with previous results that suggest that only the quality of the transferred blastocyst may be predictive of the subsequent clinical outcome, whereas morphological aspects at day 2 or day 3 have limited interest. Nevertheless, Silber (2014), during the Ovarian Club 2014 meeting, reported the results of a study conducted in a similar manner to our study. They compared implantation rate and CPR after good-quality blastocysts transfers in patients younger than 35 years, according to embryo quality at day 3 only. Finally, this study yielded conflicting results, as blastocysts arising from poor-quality embryos displayed lower implantation rate and CPR compared with good-quality embryos (39% versus 73%; $P < 0,01$; and 34% versus 54%; $P = 0.02$, respectively) (Silber, 2014). These discrepancies between our results and those reported by Silber (2014) could partly be explained by a different definition of good-quality embryos. Indeed, in the study by Silber (2014), only embryos having six to eight cells of equal size and less than 20% fragmentation were considered as good-quality embryos, whereas in our IVF unit, as defined in the 'Materials and methods' section, embryos with six to 10 blastomeres are usually quoted as good-quality embryos. Moreover, the standard microscopic scoring of embryo morphology remains necessarily a subjective method, often subject to a wide inter and intra-observer variability (Baxter Bendus et al., 2006; Paternot et al., 2011), and represents one of the main weaknesses of this study.

In order to minimize these variations, and above all to exploit innovative markers of embryo development, new technologies such as time lapse imaging have been developed. Morphokinetic variables are increasingly evaluated, from fertilization to cleavage and blastocyst stages, improving embryo scoring and selection, and at the same time enhancing the subsequent IVF–ICSI outcome (Adamson et al., 2015; Conaghan et al., 2013; Herrero et al., 2013; Motato et al., 2016; Wong et al., 2010). Reliability, however, of such morphokinetic markers for predicting the implantation potential of blastocysts remains a matter of debate. Some authors have analysed the timing of each early event during the development of good-quality blastocysts, depending on whether they implanted or not (Chamayou et al., 2013; Desai et al., 2014). Finally, few parameters, such as time to syngamy (tPNf), times to 2 (t2), 3 (t3), 5 (t5) and eight (t8) cells, t2-tPNF, t5-t2 (Desai et al., 2014), or cc3 (t5-t3) (Chamayou et al., 2013), were significantly different between implanted and non-implanted blastocysts. Furthermore, Motato et al. (2016) have recently built a hierarchical classification of blastocysts according to the following morphokinetic parameters: (i) the time of morulation (tM), and (ii) timing of transition from 5-blastomere embryo to until eight-blastomere embryo (t8–t5). In this study, they demonstrated the capacity of these variables to predict the ability of obtained blastocysts to implant. Clearly, either a wide variation between these implantation-associated markers or the lack of validation on an external independent series can be pointed out, probably because of the limitations of these studies, in particular the retrospective design and the limited number of enrolled blastocyst

transfers. Yet, the value of morphokinetic patterns, helping to select good-quality blastocysts, needs to be further evaluated in a prospective way.

The present study, however, presents several limitations deserving discussion. First, the retrospective nature and the number of included cycles necessarily affect the statistical power of the present investigation, and no formal conclusion should be drawn from these results before proceeding to a prospective randomized study that would confirm them. Over a 4-year period, only 291 good-quality blastocyst transfers have been conducted in our IVF unit. In fact, embryo vitrification, authorized by the French legislation since the end of 2010, was concomitant and required a preliminary evaluation before its general implementation for embryos both at cleavage and blastocyst stages. As a consequence, the improvement of embryo culture strategy in our centre has been delayed. Moreover, only blastocyst transfers with known implantation data could have been included, excluding heterogeneous transfers of two blastocysts (one good-quality and one poor-quality blastocyst), or double blastocyst transfers displaying a 50% implantation rate, thus reducing the number of useable cycles. Therefore, fresh and cryopreserved blastocyst transfers were deliberately included, enabling in this way a consequent increase in the number of analysed transfers. In fact, the freeze-all policy was used, notably in case of elevated serum progesterone level on the day of ovulation triggering or in patients at high risk of ovarian hyperstimulation syndrome, so that the cycle outcomes for such patients were not compromised. Yet, a recent study reported similar clinical outcomes after fresh or vitrified-warmed blastocyst transfers (Roy et al., 2014), suggesting that the inclusion of vitrified-warmed blastocyst transfers might not affect the outcomes in either of our study groups. Finally, male factor infertilities were not excluded from this analysis, as the euploidic status of day 5 blastocysts, when available, and consequently their potential to implant, might not be impaired by semen alterations, as suggested by several authors during the ASRM Congress in 2016 (unpublished data).

In the light of the present findings, the embryologist may be encouraged to no longer assess embryo quality at day 2 or day 3 in case of embryo culture strategy. In addition, avoiding unnecessary retrievals of culture dishes from the incubator could reduce deleterious stresses on embryos without jeopardizing blastulation or implantation rate (Zhang et al., 2010).

In conclusion, good-quality blastocyst transfer should be conducted irrespective of embryo quality at cleavage stage, as it may not compromise success rates in a good-prognosis population. A prospective randomized study, when at least two similar good-quality blastocysts arising from both a good-quality embryo and a poor-quality embryo at day 2 or day 3 are available at day 5, would be the only way to accurately answer the question raised. Furthermore, other non-invasive methods, such as the use of morphokinetic parameters, could represent valuable tools to predict the blastocyst implantation potential, and need further evaluation.

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