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Obstetric and perinatal outcomes of singletons after single blastocyst transfer: is there any difference according to blastocyst morphology?

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KEY MESSAGE
It has been shown that blastocyst transfer with poor morphology is associated with reduced implantation. Our results seem to be reassuring, as the transfer of a single morphologically poor blastocyst did not have a deleterious effect on obstetric or perinatal outcome compared with a blastocyst with good or fair morphology.

A B S T R A C T

A strong correlation between blastocyst morphology and implantation has been shown by many studies. The consequences and effects of assisted reproductive techniques on children's short and long-term health have always been a source of discussion. The obstetric and perinatal outcome of singletons according to blastocyst morphology has rarely been evaluated. The aim of this observational study is to determine whether a relationship exists between blastocyst morphology and obstetric and perinatal outcomes. A total of 799 singleton clinical pregnancies were analysed after transfer of a single fresh blastocyst on day 5 between 2006 and 2013. Blastocysts were divided into four groups based on their morphology on day 5: group 1 = good morphology blastocysts; group 2 = fair morphology blastocysts; group 3 = poor morphology blastocysts and group 4 = early (B1/B2) blastocysts. Obstetric and perinatal outcomes were compared between the four groups. After adjustment for some confounding variables, main obstetric and perinatal outcomes after transfer of blastocysts with poor morphological characteristics were not associated with increased adverse obstetric and perinatal events. Sex ratio was significantly higher in group 1 compared with groups 2, 3 and 4, and in Group 2 compared with Group 3 ($P < 0.001$) even after adjustment ($P < 0.05$).

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Introduction

Blastocele expansion, organization of inner cell mass (ICM) and trophectoderm cells are used to evaluate blastocyst quality (Gardner and Schoolcraft, 1999). A strong correlation between blastocyst morphology and implantation has been demonstrated by many studies (Balaban et al., 2000, 2006; Goto et al., 2011; Guerif et al., 2010). Which of the morphological characteristics is the strongest predicting factor for success after blastocyst transfer, however, is still a matter of debate (Ahlström et al., 2011; Hill et al., 2013; Honnma et al., 2012; Richter et al., 2001; Thompson et al., 2013; Van den Abbeel et al., 2013).

The main goal of IVF is the birth of a single healthy child. Indeed, the consequences and the effects of assisted reproductive techniques on children's short- and long-term health have always been a source of discussion. There seems to be an increased risk of prematurity, low birth weight and neonatal mortality among singletons born after assisted reproduction techniques (Ceelen et al., 2008; McDonald et al., 2009; Pinborg et al., 2013; Reddy et al., 2007) compared with the general population. Moreover, some studies have reported an increase in birth defects after IVF (Hansen et al., 2013; Pandey et al., 2012; Wen et al., 2012). It has also been reported that singleton pregnancies resulting from assisted reproduction techniques are at increased frequency of maternal complications (Jackson et al., 2004; Poikkeus et al., 2007; Reddy et al., 2007). It has not been elucidated to date whether such outcomes might be attributable to certain aspects of assisted reproduction techniques itself or to patient infertility (De Geyter et al., 2006; Hayashi et al., 2012; Rimm et al., 2011; Romundstad et al., 2008; Thomson et al., 2005).

Some studies have focused on the stage of the embryo on transfer and compared obstetric and perinatal outcomes between early and late embryo stages (Dar et al, 2013, 2014; Fernando et al., 2012; Källén et al., 2010; Kalra et al., 2012; Oron et al., 2014a, 2015). In some of these studies, preterm singleton birth rates (<37 weeks) (Dar et al, 2013, 2014; Källén et al., 2010; Kalra et al., 2012) and congenital malformation rates (Dar et al., 2014; Källén et al., 2010) were increased after blastocyst transfer compared with cleavage-stage transfer. By contrast, other studies showed no difference between blastocyst transfer and cleavage-stage transfer (Fernando et al., 2012). Detailed embryo morphology, however, was not investigated in any of these studies.

To the best of our knowledge, only a Canadian group has analysed the perinatal outcome of singletons according to blastocyst morphology (Oron et al., 2014a, 2014b, 2015). The number of live births after the transfer of a single poor-quality blastocyst, however, was low (n = 23), and little information was available about obstetric outcome when blastocyst morphology was considered separately. The aim of our study was to evaluate obstetric and perinatal outcomes in four groups defined according to more detailed morphological characteristics (good, fair, poor and early stage) of the single blastocyst transferred. We did not find increased rates of adverse obstetric and perinatal outcomes after transfer of blastocysts with morphologically poor characteristics.

Materials and methods

Study design

This observational study was undertaken at the IVF Unit, Bretonneau University Hospital, Tours, France, between January 2006 and December 2013. The inclusion criteria comprised the following: couples attempting first or second IVF; couples with a single fresh blastocyst transferred on day 5; attempts using non-donor oocytes; and couples achieving a singleton clinical pregnancy.

All couples were given clear information by a physician on the theoretical disadvantages (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. Couples were included in the study only once.

After each birth, a questionnaire was sent to the doctor who delivered the patient with questions on pregnancy and perinatal outcomes. It was not possible to obtain pregnancy outcome for 17 patients (2%). Reports of all patients were then regularly updated in our database in accordance with our usual practice. For the patients with a known live birth (n = 651), the questionnaire was completed for 557 patients (85.6%). Trained members of our staff telephoned the obstetrician to obtain missing data for the remaining 94 patients (14.4%) for whom information was lacking.

Ethics statement

It is current practice in our IVF centre to transfer one blastocyst on day 5 for couples attempting first or second IVF, independently of embryo quality on day 2. All participating couples had provided written informed consent to have the study results reported and published. The protocol for this observational study was approved by the Ethics Committee on Research involving Human Subjects of our hospital on 13 October 2016 (Research Project No 2016 065).

IVF procedure

The ovarian stimulation protocol and the main IVF and intracytoplasmic sperm injection procedures used have already been described elsewhere (Guerif et al., 2004). Briefly, embryo culture with sequential media and assessment were carried out as follows: fertilization (day 0) was performed in Sydney IVF Fertilization medium™ (Cook, Brisbane, Australia). The following morning (day 1), the oocytes were individually placed in microdrops (25 µl) in Sydney IVF Cleavage medium™ (Cook, Brisbane, Australia) under Sydney IVF culture oil™ (Cook, Brisbane, Australia). From day 3 to day 5/6, single embryo culture was carried out in microdrops in Sydney IVF Blastocyst medium™ (Cook, Brisbane, Australia) under Sydney IVF culture oil™ (Cook, Brisbane, Australia). All cultures took place in K-Minc incubators™ (Cook, Brisbane, Australia) at 37°C with 6% CO_2, 5% O_2 and 89% N_2.

Assessment of blastocyst morphology

All the subsequent optical assessments were carried out using an inverted microscope with Hoffman modulation contrast (×200 and ×400 magnification). For all couples included in the study, the whole cohort was placed in extended embryo culture with the intention to transfer a single blastocyst. The outcome of extended embryo culture was recorded for each individually cultured embryo. The morphological assessment was based on the expansion of the blastocoele cavity (B1–B6) and the number and cohesiveness of the inner cell mass (ICM) and trophectodermal cells (Gardner and Schoolcraft, 1999). When an embryo had started to expand, i.e. for blastocysts graded as 3–6 (full blastocysts onwards), it was then possible to assign independent scores

to the ICM and the trophectoderm (Balaban et al., 2011). Blastocyst grading was carried out by four trained embryologists over the 8-year period, with a double evaluation for each observation. Four groups have been defined as follows: group 1 comprised morphologically good blastocysts (B3–B6 on Day 5 and with at least A for the ICM or the trophectoderm and at worst B for the other part [*n* = 313]). Morphologically fair blastocysts (B3–B6 on day 5 and with B for ICM and B for the trophectoderm [*n* = 254]) were placed in group 2. Group 3 consisted of morphologically poor blastocysts (B3–B6 on day 5 and with at least C for ICM or C for the trophectoderm [*n* = 141]) and group 4 comprised B1 and B2 blastocysts (*n* = 91). As B1 and B2 blastocysts have a slightly expanded blastocoele, both ICM and trophectodermal cells are not assessed at these specific stages (Gardner and Schoolcraft, 1999). As it is not possible to predict at early blastocyst stage whether the morphology would become good, fair or poor, B1 and B2 blastocysts have been considered separately in group 4. Supernumerary blastocysts at the B3–B6 stages on day 5 or on day 6 with A/B ICM and A/B trophectoderm were frozen according to the cryopreservation procedures that have already been described (Guerif et al., 2004). Frozen blastocysts have not been included in the study.

Clinical outcome

First, rates of clinical pregnancy and live birth after transfer of a single fresh blastocyst on day 5 were calculated in couples attempting first or second IVF and using non-donor oocytes. Clinical pregnancy was defined as the presence of a gestational sac with heart activity on ultrasound examination 5 weeks after oocyte retrieval. Then, in couples included in the study (couples achieving a singleton clinical pregnancy), miscarriage, ectopic pregnancy, therapeutic abortion, intrauterine fetal death and live birth rates were assessed. Pregnancy outcomes, including maternal complications and adverse neonatal outcomes, were recorded for pregnancies resulting in a live birth. Gestational duration was determined by subtraction of the oocyte retrieval date from the date of birth and the addition of 14 days.

Adverse pregnancy outcomes (regardless of the trimester of occurrence) included pregnancy bleeding, gestational diabetes, pregnancy-induced hypertension, preeclampsia, premature rupture of membranes, placenta previa, placental abruption, preterm labour (uterine contractions and significant cervical changes before 37 weeks of gestation), maternal hospitalization and postpartum haemorrhage. The sex ratio, defined as the ratio of males to females, was determined for each group. The neonatal complications recorded were as follows: low birth weight (LBW) defined as birthweight less than 2500 g (whatever the gestational age), LBW after 37 weeks of gestation, small for gestational age (SGA) and very SGA defined as birthweight below the 10th percentile and below the third percentile for gestational age, respectively (using the French population-based liveborn infant birthweight curves), large for gestational age (LGA) and very LGA (birthweight above the 90th percentile and the 97th percentile, respectively), preterm delivery less than 37 weeks of gestation and early preterm delivery less than 32 weeks of gestation, neonatal complications such as admission to the neonatal Intensive Care Unit or neonatal death and congenital malformations (detected during pregnancy or the neonatal period).

Statistical analysis

Statistical analysis was conducted using Statview 4.1 software (Abacus Concepts, Berkeley, CA, USA). Quantitative variables were compared by analysis of variance using the Student's t-test. If necessary, a Kruskal–Wallis test was applied. Data were expressed as mean ± SD. Qualitative data were compared using chi-square test. The Fisher's exact test was used to compare small samples sizes. The results were analysed after adjustment for some confounding variables (maternal age, body mass index, parity, smoking and gender of the baby). Differences were considered significant when $P < 0.05$.

Results

In the study period, 1766 cycles with a single fresh blastocyst on day 5, in couples attempting first or second IVF and using non-donor oocytes were realized. Clinical pregnancy and live birth rates per transfer were significantly higher in group 1 compared with the other groups (**Table 1**). Both rates decreased with deteriorating morphology ($P < 0.001$).

The inclusion and exclusion criteria for our study are presented in the flow chart (**Figure 1**). Among the 841 couples achieving a clinical

Table 1 – Outcomes of cycles with transfer of a single fresh blastocyst on day 5 in couples attempting first or second IVF and using non-donor oocytes and pregnancy outcomes according to morphology of the transferred blastocyst.

	Total	Group 1: good morphology	Group 2: fair morphology	Group 3: poor morphology	Group 4: early stages	Overall *P*-value
Number of transfers of a single blastocyst	1766	594	557	349	266	/
Clinical pregnancy rate per transfer[e]	841	330 (55.6)[b,c,d]	263 (47.2)[b,e]	152 (43.6)[c]	96 (36.1)[d,e]	<0.001
Live birth rate per transfer[f]	691	278 (46.8)[b,c,d]	217 (39.0)[b,e]	119 (34.1)[c]	77 (28.9)[d,e]	<0.001
Number of singleton clinical pregnancies included in the study	799	313	254	141	91	/
Miscarriages (%)	124 (15.5)	43 (13.7)	37 (14.6)	27 (19.1)	17 (18.7)	NS
Ectopic pregnancies (%)	10 (1.3)	3 (1.0)	4 (1.6)	3 (2.1)	0 (0)	NS
Therapeutic abortions (%)	6 (0.8)	1 (0.3)	2 (0.8)	2 (1.4)	1 (1.1)	NS
Intrauterine fetal deaths (%)	8 (1.0)	5 (1.6)	2 (0.8)	0 (0)	1 (1.1)	NS
Live births (%)	651 (81.5)	261 (83.4)	209 (82.3)	109 (77.3)	72 (79.1)	NS

[a] Data are presented as numbers (%).
[b–e] Values with the same superscript are significantly different ($P < 0.05$).
[f] Inclusion of single and twin pregnancies.
NS, not statistically significant.

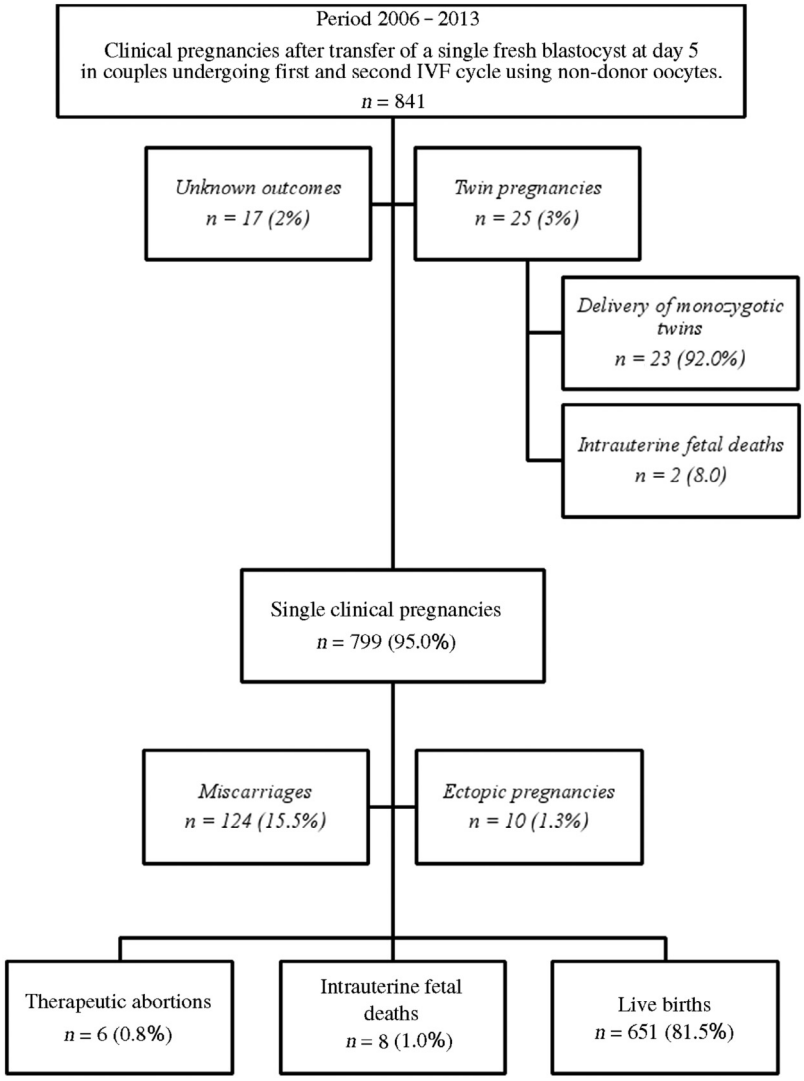

Figure 1 – Flow chart of the study.

pregnancy, 799 couples met the inclusion criteria. Twenty-five twin pregnancies (3%) and 17 pregnancies whose outcomes were unknown (2%) were excluded.

Of the 799 singleton clinical pregnancies initiated in the study period, 313 pregnancies were allocated to group 1, 254 to group 2, 141 to group 3 and 91 to group 4. The numbers and rates of miscarriages, ectopic pregnancies, therapeutic abortions, intrauterine fetal deaths and live births are reported in **Table 1**, with no difference between groups. The numbers of pregnancies ending in a live birth were 261 (83.4%), 209 (82.3%), 109 (77.3%) and 72 (79.1%) for groups 1, 2, 3 and 4, respectively. The reasons for therapeutic abortions were as follows: group 1 (n = 1), Trisomy 21; group 2 (n = 2), Trisomy 21 in two singletons; group 3 (n = 2), Trisomy 18 for one singleton, chorionic haematoma and premature rupture of membranes with anamnios at 12–15 weeks of gestation for the second singleton; and group 4 (n = 1), superior celosomia. The reasons for intrauterine fetal death were as follows: group 1 (n = 5), chronic circulatory failure of the placenta, nuchal cord, multiple fetal malformations (dolichocephaly and ventricular septal defect) and intrauterine growth retardation, oligoamnios in the second trimester and placental ischaemia, Factor V Leiden homozygous mutation discovered in the mother; group 2 (n = 2), placenta abruption followed by premature rupture of membranes at 22 weeks of gestation, major hypotrophy of the placenta; and group 4 (n = 1), placental fetal thrombosis.

Epidemiological data and cycle characteristics

Patient characteristics are shown in **Table 2**. Maternal age was significantly higher in group 3 than in groups 1 and 2 (P = 0.009). All other parameters, including diagnosis of infertility, were similar in all four groups.

The clinical and biological data of the cycles are presented in **Table 3**. No difference was found in the total dose of FSH used for the stimulation. The total number of mature oocytes, however, was higher in group 1 compared with groups 3 and 4, and in group 2 compared with group 4 (P < 0.001). This was related to higher numbers of oocytes collected, whereas the rate of mature oocytes was not different between groups. Moreover, group 1 had significantly more embryos on day 2 and higher rates of blastulation and frozen blastocysts compared with the other three groups (P < 0.001 for each parameter). Similarly, group 2 had a significantly higher frozen blastocyst rate compared with groups 3 and 4 (P < 0.001).

Table 2 – Epidemiological data according to the morphology of the transferred blastocyst in cycles achieving a singleton clinical pregnancy after transfer of a single blastocyst on day 5.[a]

	Group 1 pregnancies from 'good morphology' (n = 313)	Group 2 pregnancies from 'fair morphology' (n = 254)	Group 3 pregnancies from 'poor morphology' (n = 141)	Group 4 pregnancies from 'early stages' (n = 91)	*P*-value
Maternal age (years)	30.7 ± 4.1[b]	30.9 ± 4.9[c]	32.1 ± 4.2[b,c]	31.1 ± 4.3	0.009
Paternal age (years)	33.4 ± 5.5	33.9 ± 5.9	34.7 ± 5.4	34.5 ± 6.0	NS
Maternal body mass index	23.4 ± 4.5	23.9 ± 4.8	24.2 ± 4.9	23.3 ± 4.0	NS
Maternal smoking (%)	110 (35.1)	81 (31.9)	44 (31.2)	36 (39.6)	NS
Duration of infertility (years)	4.4 ± 2.4	4.7 ± 2.0	4.9 ± 2.9	4.7 ± 2.8	NS
Primiparous (%)	156 (49.8)	154 (60.6)	71 (50.4)	49 (53.8)	NS
Cause of infertility (%)					
Female factor	98 (31.3)	72 (28.3)	35 (24.8)	22 (24.2)	NS
Male factor	45 (14.4)	47 (18.5)	26 (18.4)	17 (18.7)	
Mixed	158 (50.5)	132 (52.0)	75 (53.2)	49 (53.8)	
Idiopathic	12 (3.8)	3 (1.2)	5 (3.5)	3 (3.3)	
Female factor (%)					
Ovulatory factor	215 (68.7)	176 (69.3)	96 (68.1)	65 (71.4)	NS
Tubal factor	83 (26.5)	56 (22.0)	35 (24.8)	15 (16.5)	NS
Endometriosis	38 (12.1)	23 (9.1)	15 (10.6)	10 (11.0)	NS

[a] Data are presented as mean ± SD and numbers (%).
[b,c] Values with the same superscript are significantly different ($P < 0.05$).
NS, not statistically significant.

Obstetric outcomes

The obstetric complications for live births after transfer of a single blastocyst with different morphological characteristics are presented in **Table 4**. Almost all items analysed were similar between the groups, except for the frequency of pregnancy bleeding and preterm labour which were significantly higher in Group 2 than in Groups 1, 3 and 4 ($P = 0.01$ and $P = 0.002$, respectively). After adjustment, the rate of bleeding remained higher in group 2 compared with group 3 and in group 2 compared with group 4, but did not reach significance between groups 1 and 2 (**Table 5**). After adjustment, the rate of preterm labour remained higher in group 2 compared with group 1 and in group 2 compared with group 3, but did not reach significance between groups 2 and 4 (**Table 5**). The modes of delivery were similar between the four groups (**Table 4**).

Perinatal outcomes

Perinatal outcomes were almost identical to all groups (**Table 6**). In fact, no difference was observed in mean birthweight, mean birth length, rates of LBW, LBW after 37 weeks of gestation, SGA, very SGA, LGA, and very LGA between the four groups. Only gestational age was significantly higher in group 3 compared with group 2 (39.8 ± 1.8 versus 39.2 ± 2.3, $P = 0.03$) and remained significant after adjustment, without significant difference between other groups (**Table 5**). All groups had identical neonatal complications and congenital malformations.

Table 3 – Clinical and biological characteristics according to the morphology of the transferred blastocyst in cycles achieving a singleton clinical pregnancy after transfer of a single blastocyst on day 5.[a]

	Group 1: pregnancies from 'good morphology' (n = 313)	Group 2: pregnancies from 'fair morphology' (n = 254)	Group 3: Pregnancies from 'poor morphology' (n = 141)	Group 4: pregnancies from 'early stages' (n = 91)	*P*-value
Total dose of FSH (IU)	1663 ± 923	1839 ± 2374	1781 ± 1012	1697 ± 835	NS
Intracytoplasmic sperm injection (%)	190 (60.7)	167 (65.7)	97 (68.8)	64 (70.3)	NS
Number of total oocytes	10.6 ± 5.3[b,c]	10.1 ± 4.7[d]	9.2 ± 3.7[b]	8.4 ± 3.7[c,d]	<0.001
Number of mature oocytes	8.3 ± 4.5[b,c]	7.7 ± 3.6[d]	6.9 ± 2.8[b]	6.6 ± 2.8[c,d]	<0.001
Mature oocyte rate (%)	82 ± 39	78 ± 18	78 ± 19	87 ± 58	NS
Fertilization rate (%)	85 ± 19	82 ± 22[b]	87 ± 26[b,c]	80 ± 21[c]	0.049
Number of total embryos on day 2	6.8 ± 3.9[b,c,d]	6.1 ± 2.9[b,e]	5.9 ± 2.4[c]	5.1 ± 2.1[d,e]	<0.001
Number of embryos in extended culture	6.1 ± 3.4[b,c,d]	5.5 ± 2.6[b,e]	5.3 ± 2.2[c,f]	4.4 ± 1.9[d,e,f]	<0.001
Number of total blastocysts	4.7 ± 2.7[b,c,d]	3.8 ± 1.9[b,e]	3.4 ± 1.7[c]	2.9 ± 1.8[d,e]	<0.001
Blastulation rate (%)	77 ± 20[b,c,f]	71 ± 23[b,e]	67 ± 24[c]	65 ± 23[d,e]	<0.001
Number of frozen blastocysts	2.6 ± 1.9[b,c,d]	1.7 ± 1.5[b,e,f]	1.3 ± 1.4[c,e,g]	0.9 ± 1.3[d,f,g]	<0.001
Frozen blastocyst rate (%)	51 ± 24[b,c,d]	39 ± 26[b,e,f]	31 ± 29[c,e,g]	22 ± 27[d,f,g]	<0.001

[a] Data are presented as mean ± SD and numbers (%).
[b–g] Values with the same superscript are significantly different ($P < 0.05$).

Table 4 – Obstetric complications according to the morphology of the transferred blastocyst in cycles resulting in a live birth after transfer of a single blastocyst on Day 5.[a,b]

	Group 1: live births from 'good morphology' (n = 261)	Group 2: live births from 'fair morphology' (n = 209)	Group 3: live births from 'poor morphology' (n = 109)	Group 4: live births from 'early stages' (n = 72)	*P*-value
Pregnancy bleeding (%)	23 (8.8)[b]	32 (15.3)[b,c,d]	6 (5.5)[c]	3 (4.2)[d]	0.01
Preimplantation genetic diagnosis (%)	19 (7.3)	16 (7.7)	16 (14.7)	4 (5.6)	NS
Gestational diabetes (%)	17 (6.5)	11 (5.3)	6 (5.5)	6 (8.3)	NS
Pregnancy-induced hypertension (%)	21 (8.0)	10 (4.8)	6 (5.5)	4 (5.6)	NS
Pre-eclampsia (%)	12 (4.6)	5 (2.4)	1 (0.9)	1 (1.4)	NS
Premature rupture of membranes (%)	13 (5.0)	10 (4.8)	5 (4.6)	3 (4.2)	NS
Placenta previa (%)	5 (1.9)	13 (6.2)	4 (3.7)	2 (2.8)	NS
Placenta abruption (%)	6 (2.3)	3 (1.4)	2 (1.8)	0 (0.0)	NS
Preterm labour (%)	18 (6.9)[b]	32 (15.3)[b,c,d]	4 (3.7)[c]	4 (5.6)[d]	0.002
Hospitalisation (%)	45 (17.2)	53 (25.4)	19 (17.4)	13 (18.1)	NS
+ Length(days)	7.3 ± 7.6	6.5 ± 7.1	4.0 ± 5.2	8.4 ± 8.2	NS
Intrauterine growth retardation (%)	8 (3.1)	12 (5.7)	6 (5.5)	3 (4.2)	NS
Postpartum haemorrhage (%)	10 (3.8)	14 (6.7)	3 (2.8)	3 (4.2)	NS
Induced labour (%)	48 (18.4)	39 (18.7)	28 (25.7)	15 (20.8)	NS
Mode of delivery (%):					
Normal vaginal delivery[e]	200 (76.6)	159 (76.1)	82 (75.2)	54 (75.0)	NS
Cesarean section before labour[f]	24 (9.2)	24 (11.5)	15 (13.8)	5 (6.9)	
Emergency Cesarean section after labour onset	37 (14.2)	26 (12.4)	12 (11.0)	13 (18.1)	

[a] Data are presented as mean ± SD and numbers (%).
[b–d] Values with the same superscript are significantly different ($P < 0.05$)
[e] Instrumental delivery included.
[f] Planned or emergency Cesarean section.
NS, not statiscally significant

Congenital defects were classified as minor or major malformations according to the EUROCAT (European Surveillance of Congenital Anomalies) classification ('EUROCAT-Guide-1.4-Section-3.2.pdf,' n.d.). Major malformations were seen in six singletons (two in group 1, two in group 2, one in group 3 and one in group 4), and minor malformations were seen in nine singletons (three in group 1, two in group 2, two in group 3 and two in group 4). The major malformations were as follows: group 1 (*n* = 2), ectrodactyly and ventricular septal defect; group 2 (*n* = 2), ventricular septal defect and congenital hip dislocation; group 3 (*n* = 1), single ventricle; and group 4 (*n* = 1) single horseshoe kidney. Minor malformations were as follows: group 1 (*n* = 3), congenital torticollis, clubfeet, unspecified cardiac murmur and associated left ventricular hypertrophy; group 2 (*n* = 2), dysplasia of the upper pole of the right kidney with ureterocele, hydronephrosis; group 3 (*n* = 2), vocal cord palsy, arachnoid cyst and group 4 (*n* = 2), Floating thumb metacarpal, curvature of penis.

Surprisingly, the sex ratio was significantly higher in group 1 than in groups 2, 3 and 4, and in group 2 compared with group 3 ($P < 0.001$). After adjustment, the sex ratio remained higher in group 1 compared with groups 2, 3 and 4 and in group 2 compared with group 3 (**Table 5**).

Discussion

As shown in previous studies, worse morphology correlates with worse pregnancy results (Balaban et al., 2000, 2006; Goto et al., 2011; Guerif et al., 2010). In our study, we confirmed these results: clinical pregnancy and live birth rates per transfer were significantly higher in group 1 compared with the other groups. Both rates decreased with morphology. The initiation of a pregnancy with morphologically poor blastocysts could cause concern with regard to the obstetric and perinatal outcomes. In this observational study, we evaluated obstetric and perinatal outcomes after single blastocyst transfer in relation to blastocyst morphology. We did not find increased rates of adverse obstetric and perinatal outcomes after transfer of blastocysts with morphologically poor characteristics. Therefore, once a pregnancy is achieved, there is no difference in obstetric and perinatal outcomes.

The main characteristics of the populations of the four groups, such as paternal age, maternal BMI, maternal smoking, duration of infertility, parity and cause of infertility were similar. In terms of biological parameters, the results for group 3 were always lower for the number of embryos on day 2 and for rates of blastulation and frozen blastocysts than group 1. These results could be explained by higher maternal age in group 3. The total dose of FSH administered and the IVF technique used (classical IVF or ICSI), however, were identical in all groups.

Several methodological aspects support the results of our study: all the blastocysts were evaluated by the same trained embryologists in our IVF centre. As a consequence, the risk of variation in morphological blastocyst assessment was greatly reduced; in the study period, embryos were all cultured in the same Cook media, which is important to avoid bias owing to a medium effect on birthweight as previously suspected (Dumoulin et al., 2010; Nelissen et al., 2012); all pregnancies were initiated after transfer of a single blastocyst. Such a strategy has the advantage of excluding the potential vanishing twin phenomenon after double embryo transfer (Poikkeus et al., 2007); the number of live births included in the analysis (*n* = 651) allowed us to analyse the relationship between blastocyst morphology and obstetric and perinatal outcomes more accurately. A previous

Table 5 – Obstetric and neonatal outcomes adjusted for maternal age, body mass index, smoking, parity and sex of the child in cycles resulting in a live birth after transfer of a single blastocyst on Day 5.

	Group 2 : live births from 'fair morphology'		Group 3: live births from 'poor morphology'		Group 4: live births from 'early stages'		Group 3: live births from 'poor morphology'		Group 4: live births from 'early stages'		Group 4: live births from 'early stages'	
	Odds Ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
Group 1 'Good morphology'	1.00		1.00		1.00		–		–		–	
Group 2: 'Fair morphology'	–		–		–		1.00		1.00		–	
Group 3: 'Poor morphology'	–		–		–		–		–		1.00	
Pregnancy bleeding	1.77 (0.95 to 3.32)	NS	0.57 (0.18 to 1.48)	NS	0.35 (0.05 to 1.26)	NS	0.32 (0.10 to 0.81)	0.03	0.20 (0.03 to 0.70)	0.03	0.62 (0.09 to 3.00)	NS
Gestational diabetes	0.73 (0.29 to 1.72)	NS	0.59 (0.18 to 1.62)	NS	1.41 (0.43 to 3.99)	NS	0.81 (0.24 to 2.50)	NS	1.94 (0.56 to 6.17)	NS	2.39 (0.62 to 9.34)	NS
Pregnancy-induced hypertension	0.52 (0.21 to 1.22)	NS	0.52 (0.16 to 1.40)	NS	0.63 (0.14 to 2.00)	NS	0.99 (0.28 to 3.14)	NS	1.20 (0.25 to 4.45)	NS	1.22 (0.24 to 5.33)	NS
Preeclampsia	0.43 (0.12 to 1.31)	NS	0.18 (0.01 to 1.00)	NS	0.33 (0.02 to 1.77)	NS	0.41 (0.02 to 2.83)	NS	0.76 (0.04 to 5.32)	NS	1.86 (0.07 to 48.27)	NS
Premature rupture of membranes	0.91 (0.34 to 2.28)	NS	0.93 (0.24 to 2.88)	NS	0.67 (0.10 to 2.59)	NS	1.02 (0.26 to 3.42)	NS	0.73 (0.11 tp 3.07)	NS	0.72 (0.10 to 3.90)	NS
Placenta abruption	0.63 (0.09 to 3.06)	NS	1.53 (0.20 to 8.03)	NS	–	–	2.42 (0.27 to 21.37)	NS	–	–	–	-
Preterm labour	2.44 (1.26 to 4.84)	0.009	0.54 (0.12 to 1.72)	NS	0.71 (0.16 to 2.27)	NS	0.22 (0.05 to 0.67)	0.02	0.29 (0.07 to 1.00)	NS	1.32 (0.23 to 7.43)	NS
Intrauterine growth retardation	1.81 (0.67 to 5.14)	NS	1.44 (0.40 to 4.81)	NS	1.01 (0.15 to 4.39)	NS	0.80 (0.24 to 2.36)	NS	0.56 (0.08 to 2.22)	NS	0.70 (0.10 to 3.45)	NS
Postpartum haemorrhage	1.82 (0.74 to 4.63)	NS	0.52 (0.08 to 2.15)	NS	1.34 (0.29 to 4.74)	NS	0.29 (0.04 to 1.11)	NS	0.73 (0.16 to 2.46)	NS	2.55 (0.40 to 20.02)	NS
Sex ratio[a]	0.62 (0.42 to 0.89)	0.01	0.38 (0.23 to 0.60)	<0.001	0.57 (0.33 to 0.96)	0.04	0.61 (0.38 to 0.98)	0.04	0.92 (0.54 to 1.58)	NS	1.51 (0.82 to 2.79)	NS
Gestational age	–	NS	–	NS	–	NS	–	0.003	–	NS	–	NS
Preterm delivery <37 weeks	1.83 (0.90 to 3.84)	NS	0.60 (0.16 to 1.75)	NS	1.06 (0.29 to 3.09)	NS	0.33 (0.09 to 0.90)	0.048	0.58 (0.16 to 1.60)	NS	1.77 (0.40 to 7.77)	NS
Preterm delivery <32 weeks	2.29 (0.42 to 17.02)	NS	1.00 (0.04 to 11.10)	NS	–	-	0.44 (0.02 to 3.11)	NS	–	–	–	–
Low birth weight <2500g	1.22 (0.61 to 2.45)	NS	0.32 (0.07 to 1.00)	NS	0.72 (0.20 to 2.05)	NS	0.26 (0.06 to 0.80)	0.04	0.59 (0.17 to 1.68)	NS	2.27 (0.48 to 11.96)	NS
Low birth weight <2500g after 37 weeks	0.68 (0.23 to 1.91)	NS	0.17 (0.01 to 1.00)	NS	0.57 (0.08 to 2.29)	NS	0.25 (0.01 to 1.54)	NS	0.83 (0.12 to 3.78)	NS	3.28 (0.30 to 72.15)	NS
Small for gestational age: < 10th percentile	1.21 (0.73 to 2.02)	NS	1.20 (0.63 to 2.24)	NS	0.62 (0.26 to 1.35)	NS	0.99 (0.52 to 1.85)	NS	0.51 (0.21 to 1.12)	NS	0.52 (0.20 to 1.24)	NS
Large for gestational age: > 90th percentile	0.65 (0.29 to 1.37)	NS	0.96 (0.39 to 2.17)	NS	1.08 (0.38 to 2.68)	NS	1.46 (0.56 to 3.71)	NS	1.65 (0.54 to 4.58)	NS	1.13 (0.36 to 3.36)	NS
Admission to neonatal intensive care unit	1.00 (0.53 to 1.85)	NS	0.70 (0.28 to 1.59)	NS	1.04 (0.39 to 2.43)	NS	0.71 (0.28 to 1.64)	NS	1.04 (0.39 to 2.52)	NS	1.48 (0.48 to 4.41)	NS
Congenital malformations	1.09 (0.26 to 4.23)	NS	1.54 (0.30 to 6.70)	NS	2.45 (0.48 to 10.48)	NS	1.14 (0.27 to 6.65)	NS	2.25 (0.43 to 10.63)	NS	1.60 (0.28 to 9.10)	NS

Dashes signify that no odds ratio or *P*-value could be calculated.

[a] Sex ratio was adjusted for maternal age, BMI, smoking and parity.

NS, not statistially significant.

Table 6 – Child health according to the morphology of the transferred blastocyst in cycles resulting in a live birth after transfer of a single blastocyst on day 5.[a]

	Group 1: live births from 'good morphology' (n = 261)	Group 2: live births from 'fair morphology' (n = 209)	Group 3: live births from 'poor morphology' (n = 109)	Group 4: live births from 'early stages' (n = 72)	*P*-value
Sex ratio	1.6[b,c,d]	1.0[b,e]	0.6[c,e]	0.9[d]	<0.001
Gestational age (weeks)	39.5 ± 1.9	39.2 ± 2.3[b]	39.8 ± 1.8[b]	39.6 ± 1.6	0.03
Preterm delivery <37 weeks (%)	14 (5.4)	20 (9.6)	4 (3.7)	4 (5.6)	NS
Preterm delivery <32 weeks (%)	2 (0.8)	4 (1.9)	1 (0.9)	0 (0.0)	NS
Birth length (cm)	49.3 ± 2.8	48.8 ± 3.0	49.5 ± 2.8	49.1 ± 2.7	NS
Birthweight (g)	3229 ± 545	3157 ± 591	3306 ± 577	3243 ± 493	NS
Low birth weight <2500g (%)	19 (7.3)	20 (9.6)	3 (2.8)	4 (5.6)	NS
Low birth weight <2500g after 37 weeks (%)	11 (4.2)	7 (3.3)	1 (0.9)	2 (2.8)	NS
Small for gestational age <10th percentile (%)	41 (15.7)	40 (19.1)	20 (18.3)	8 (11.1)	NS
Very small for gestational age <3rd percentile (%)	22 (8.4)	17 (8.1)	5 (4.6)	2 (2.8)	NS
Large for gestational age >90th percentile (%)	23 (8.8)	12 (5.7)	10 (9.2)	6 (8.3)	NS
Very large for gestational age: > 97th percentile (%)	4 (1.5)	2 (1.0)	4 (3.7)	0 (0.0)	NS
APGAR score 1 min	9.1 ± 1.6	9.0 ± 1.8	9.3 ± 1.8	9.1 ± 1.6	NS
APGAR score 5 min	9.8 ± 0.6	9.8 ± 0.6	9.8 ± 0.7	9.8 ± 0.6	NS
Neonatal complications (%)					
Admission to neonatal intensive care unit	31 (11.9)	24 (11.5)	10 (9.2)	8 (11.1)	NS
Neonatal death	1 (0.4)	0 (0.0)	1 (0.9)	0 (0.0)	NS
Congenital malformations (%)	5 (1.9)	4 (1.9)	3 (2.8)	3 (4.2)	NS
Minor malformations	3/5	2/4	2/3	2/3	
Major malformation	2/5	2/4	1/3	1/3	

[a] Data are presented as mean ± SD and numbers (%).
[b–e] Values with the same superscript are significantly different ($P < 0.05$).
APGAR, appearance, pulse, grimace, activity, respiration.

study (Oron et al., 2014b) analysed obstetric and perinatal outcomes as a function of blastocyst morphology according to criteria described initially by Gardner (Gardner and Schoolcraft, 1999). In our study, owing to the inclusion of a higher number of patients, we were able to analyse more precisely the effects of blastocyst morphology by subdividing the good-quality blastocyst group defined by Oron et al. (2014b) into two groups: one group (group 1) including morphologically good blastocysts (≥B3 A/A or B/A or A/B) and another group (group 2), including morphologically fair blastocysts (≥B3 B/B). In the study by Oron et al. (2014b), a blastocyst lower than 3BB quality was defined as poor quality. In our study, a blastocyst defined as at least stage B3 with grade C for inner cell mass and/or trophectoderm, was classified as a morphologically poor blastocyst (group 3), whereas early stages (B1/B2) were considered separately (group 4). In fact, both inner cell mass and the trophectoderm could be evaluated precisely in this last group (Gardner and Schoolcraft, 1999). In our IVF centre, we routinely transfer a single blastocyst to couples undertaking their first or second IVF attempt, regardless of embryo morphology on day 2 (Guerif et al., 2009). Therefore, although the number of transfers leading to a pregnancy after transfer of a morphologically poor blastocyst ($n = 141$) was four times lower than transfer of a morphologically good/fair blastocyst ($n = 567$), the number of participants remained significantly higher compared with the group in the study by Oron et al. (2014b) ($n = 23$). It has previously been shown that perinatal outcome may be improved in transfers of cryopreserved embryos (Maheshwari et al., 2012; Pelkonen et al., 2010). Therefore, only fresh blastocyst transfers were included in the study to avoid any bias originating from frozen-thawed blastocysts.

Many studies have shown a correlation between blastocyst morphology and success in implantation, clinical pregnancy and live birth rates (Balaban et al., 2000, 2006; Goto et al., 2011; Guerif et al., 2010). It has still not been clearly established, however, which of the morphological characteristics (expansion, trophectoderm or inner cell mass) of a blastocyst is the strongest factor predictive of success after transfer. Altogether, transfer of single blastocysts with poor morphology has been reported to be associated with decreased rates of implantation (Balaban et al., 2000, 2006; Goto et al., 2011; Guerif et al., 2010). Once a clinical pregnancy was achieved, however, we showed that the live birth rate was not significantly different whatever the morphology of the blastocyst transferred, ranging from 77.3% (group 3) to 83.4% (group 1). We only observed a trend towards a higher rate of miscarriages, both in groups 3 and 4 without reaching statistical significance. These results were in agreement with a previous study (Oron et al., 2014b).

It has been reported that singleton assisted reproduction technique pregnancies had increased rates of maternal complications, including gestational diabetes, preeclampsia, placenta previa, placental abruption, caesarean delivery and maternal hospitalization (Jackson et al., 2004; Poikkeus et al., 2007; Reddy et al., 2007). In our study, preterm labour was significantly more frequent only in group 2 ($P = 0.002$) but we did not find any difference in preterm delivery rate between the four groups. The mean gestational age in group 2 (39.2 ± 2.3 weeks) was only significantly lower compared with group 3 (39.8 ± 1.8 weeks). In terms of obstetric complications, only the pregnancy bleeding rate was significantly higher in group 2 compared with groups 1, 3 and 4 ($P = 0.01$), and in terms of neonatal outcome, there was no difference in mean birth weight, rates of low birth weight, neonatal mortality or congenital malformations between the four groups. The overall malformation rate (2.3%) may seem low compared with other studies. It could be partly explained by the fact that, in our study,

only the malformations detected at birth (live births) were included. By contrast, other studies included congenital malformations from pregnancy terminations with a gestation period of at least 20 weeks (Davies et al., 2012) or detected in the years after the birth (Davies et al., 2012; Yin et al., 2013). The size of groups does not allow us to draw any conclusion about the trend towards a higher rate of congenital malformations (without reaching statistical significance) in group 3 (poor morphology) and group 4 (early stages) and further studies are necessary. The results were the same after adjusting for maternal confounding variables (maternal age, body mass index, parity, smoking) and gender of the baby.

Surprisingly, the sex ratio was significantly higher in group 1 than in groups 2, 3 and 4, and in group 2 compared with group 3 ($P < 0.001$). Some studies have reported differences in the sex ratio after blastocyst culture, with a higher male to female ratio (Bu et al., 2014; Chang et al., 2009; Maalouf et al., 2014). Moreover, Alfarawati et al. (2011) showed that male embryos developed significantly faster than females, and using time-lapse technology. Bronet et al. (2015) found that two specific kinetic variables were able to predict a female embryo (timing between division to three cells and division to four cells [> 2 h] and timing of morula formation [80.8–90.9 h]). In our current practice, we promote blastocyst transfer with the highest speed of development (=highest expansion rate on day 5). Such a strategy could thus favour transfer of male embryos because of faster development of such embryos compared with female embryos and could partially explain the sex ratio observed in our study. Some investigators, however, did not find any variation in sex ratio after blastocyst transfer (Csokmay et al., 2009) and others did not find any difference between male and female embryos in the morphokinetic development patterns from fertilization to implantation (Serdarogullari et al., 2014).

It has been shown that blastocyst transfer with poor morphology is associated with reduced implantation. The initiation of a pregnancy in such conditions could cause concern for some couples for obstetric and perinatal outcomes. Our results seem to be reassuring for such IVF couples, as the transfer of a single morphologically poor blastocyst did not have a deleterious effect on obstetric or perinatal outcome compared with a blastocyst with good or fair morphology.

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