EMBRYO BIOLOGY

Developmental capacity and pregnancy rate of tetrahedral- versus non-tetrahedral-shaped 4-cell stage human embryos

G. Cauffman · G. Verheyen · H. Tournaye ·
H. Van de Velde

Abstract

Purpose The arrangement of the blastomeres within the 4-cell stage embryo reflects the orientation of the cleavage planes during the second division. To examine their relevance, the developmental capacity and the pregnancy rate were compared between tetrahedral-shaped and non-tetrahedral-shaped 4-cell stage human embryos.

Methods The study included 3,546 4-cell stage embryos. The arrangement of the blastomeres at the 4-cell stage was annotated as being tetrahedral or non-tetrahedral on day 2 of preimplantation development. Embryo quality was compared on day 3 and day 5. Pregnancy rates were calculated per single embryo transfer on day 3 or day 5.

Results In total, 2,803 4-cell stage embryos (79 %) displayed a tetrahedral arrangement and 743 (21 %) displayed a non-tetrahedral arrangement. Tetrahedral-shaped embryos developed more into high-quality embryos on day 3 ($p<0.001$) and day 5 ($p=0.036$) and had a higher blastulation rate ($p=0.009$). Though, the number of high-quality embryos selected for transfer did not differ between both groups on day 3 ($p=0.167$) and day 5 ($p\sim1$). Three hundred thirty single embryo transfers were analysed. No significant difference in clinical pregnancy was found between both groups after transfer on day 3 ($p=0.209$) and day 5 ($p=0.653$).

Conclusions The arrangement of the blastomeres according to their previous cleavage planes was correlated to the developmental potential of the 4-cell stage embryo up to the blastocyst stage. If embryo transfers are performed on day 3 and day 5 of development using embryos of adequate quality, the blastomere arrangement at the 4-cell

stage had no predictable value regarding pregnancy success.

Keywords Embryo morphology · Blastomere arrangement · Tetrahedron · Embryo selection · Cleavage plane

Introduction

In 1975, Gulyas demonstrated that the blastomeres of 4-cell stage rabbit embryos are arranged crosswise in a tetrahedron [1]. This arrangement results of a first division along the meridional axis, followed by a second division in which one daughter cell divides meridionally and the other equatorially, perpendicular to the meridional axis (rotational cleavage) (Fig. 1) [2]. Like in other mammals, the four cells of the human embryo too are normally arranged in a tetrahedron (Fig. 2a) [3, 4]. However, in some cases, the blastomeres are organized in a non-tetrahedral way displaying a planar arrangement (Fig. 2b). This arrangement is the result of similar orientations of the cleavage planes during the second division, i.e. two consecutive meridional or equatorial divisions (Fig. 1).

Mammalian oocytes consist of an animal pole, marked by the second polar body and a vegetal pole on the opposite site [5–8]. The first meridional division occurs according to the animal-vegetal axis of the zygote [9, 10]. The two daughter blastomeres each inherit similar proportions of animal and vegetal ooplasm. During the second division, the animal and vegetal gradient is distributed differently to the four blastomeres due to the different orientations of the cleavage planes [1, 2]. The meridional division generates daughter blastomeres containing animal and vegetal ooplasm. The equatorial division generates one blastomere with mostly animal ooplasm and one with mostly vegetal ooplasm. Partitioning of the ooplasm may introduce developmentally significant asymmetries between the blastomeres at the 4-cell stage. In 1997, Edwards and Beard introduced a model of early cell determination [5]. They proposed that the two blastomeres

G. Cauffman (✉) · G. Verheyen · H. Tournaye · H. Van de Velde
Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel,
Laarbeeklaan 101, 1090 Brussels, Belgium
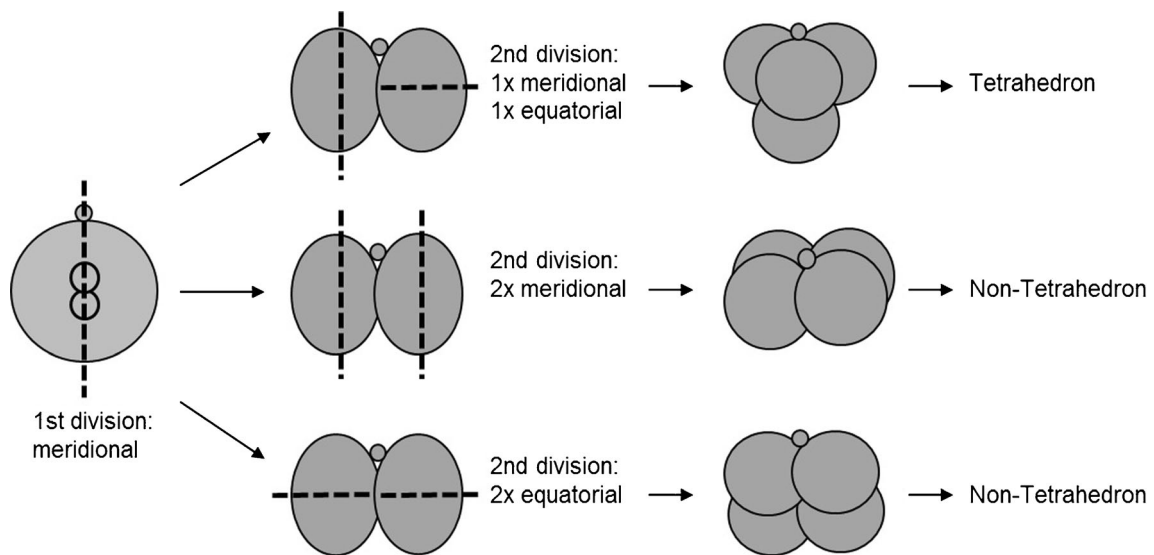e-mail: greet.cauffman@uzbrussel.be

Fig. 1 Schematic presentation of the generation of tetrahedral and non-tetrahedral-shaped embryos. The second polar body is presented by the small circle. *Dash line*: orientation of the cleavage plane

inheriting animal and vegetal ooplasm are precursors of the inner cell mass (ICM), the blastomere inheriting mainly animal ooplasm is the precursor of the trophectoderm (TE) and the blastomere inheriting mainly vegetal ooplasm is the precursor of the germ line [5, 8, 11]. This model of early asymmetry was extrapolated to the human and supported by the polarized distribution of proteins such as Leptin and STAT3 in the oocyte, their differential distribution between the blastomeres at the 4-cell stage and later on between the ICM and TE [6] and by the reciprocal expression of *OCT-4* and β*hCG* transcripts at the 4-cell stage [11, 12]. Indirect evidence of

oocyte polarity in the human was given by the correlation between the site of sperm deposition in the oocyte and the embryo development rate [13]. The early interpretation of Edwards's model that the generated differences would be deterministic proved wrong as early cells can change their fate after repositioning [14]. However, the altered orientation of the cleavage planes in non-tetrahedral-shaped embryos may cause aberrant distributions of animal and vegetal ooplasm, including cell organelles and maternally inherited transcripts and proteins, between the blastomeres [5, 6]. Whether this affects the development and

Fig. 2 Pictures of human 4-cell stage embryos on day 2 of preimplantation development. **a**. Tetrahedral-shaped embryos. **b**. Non-tetrahedral-shaped embryos
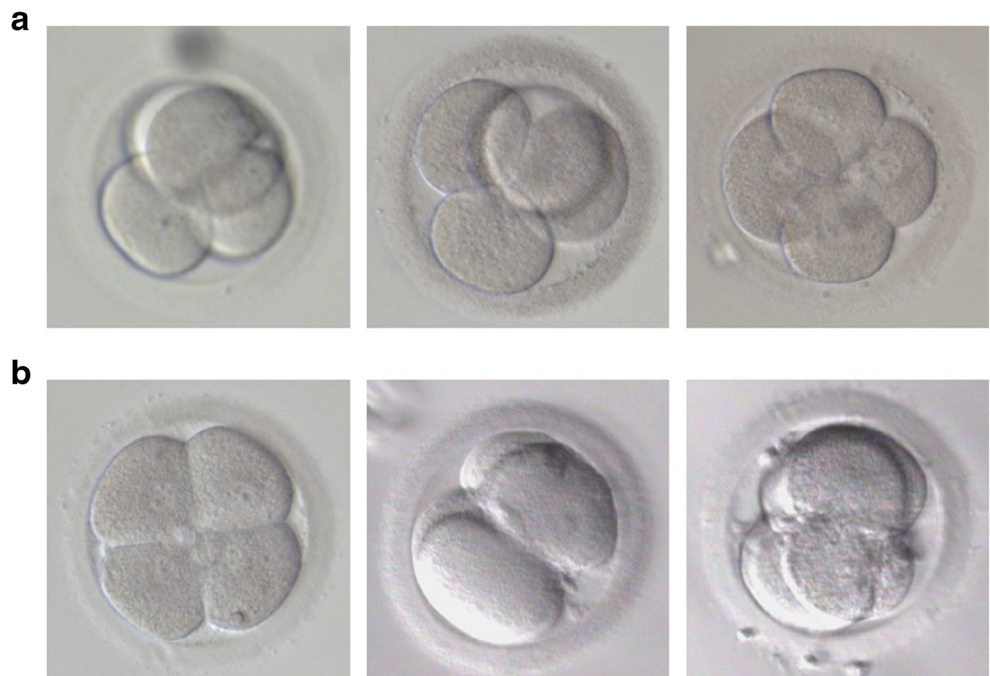
the implantation potential of non-tetrahedral-shaped 4-cell stage human embryos is unknown.

Selecting the most competent embryo within a cohort constitutes a major challenge [15, 16]. Generally, embryo assessment is limited to static microscopic evaluations [17–19]. Alternative selection methods include genetic screening [20–22], morphokinetics [23], metabolomics [24, 25], proteomics, oxygen consumption measurement and birefringence imaging [26]. The daily application of these innovating techniques is limited as they are invasive, complex, time-consuming, expensive and/or not yet proven to be superior to the static morphological evaluations, in particular compared to blastocyst culture [27]. Hence, embryo selection based on morphology remains the most common and generally accepted method to date. The spatial arrangement of the blastomeres within the embryo is currently not considered as a potential criterion for embryo selection [18, 28].

We examined whether the arrangement of the blastomeres at the 4-cell stage, as a result of the previous cleavage planes, has a value in the assessment of the developmental potential towards day 3 and day 5 of human preimplantation development and/or in the assessment of pregnancy.

Materials and methods

This study is an observational retrospective cohort study. The treatment of the patients in this study did not differ from routine assisted reproductive procedures. The study was conducted at the Centre for Reproductive Medicine at the UZ Brussel during a 3 year period.

Ovarian stimulation and oocyte retrieval

The patients underwent either natural or stimulated cycles. Ovarian stimulation was performed using urinary (Menopur, Ferring Pharmaceuticals A/S, Copenhagen, Denmark) or recombinant FSH (Puregon, NV Organon, Oss, The Netherlands; or Gonal F, Merck-Serono, Geneva, Switzerland) in combination with a GnRH antagonist (Orgalutran, NV Organon) or agonist (Suprefact, Aventis Pharma, Frankfurt, Germany) protocol. Final oocyte maturation was achieved by injection of hCG (Pregnyl, Schering-Plough, Oss, The Netherlands, or Profasi, Merck-Serono). Oocyte retrieval was carried out using vaginal ultrasound-guided puncture of ovarian follicles 36 h after hCG administration. Intravaginally administered progesterone (Utrogestan, Besins, Brussels, Belgium) was used for luteal phase supplementation.

Data collection and embryo culture

The study included 901 ICSI cycles (86.6 %), 86 IVF cycles (8.3 %) and 53 IVF vs ICSI cycles (5.1 %). Ejaculated semen was used in the majority of the cycles (93.3 %), testicular sperm was used in 6.7 % of the cycles. PGD cycles and cycles using vitrified oocytes or in vitro matured oocytes were excluded. Oocyte denudation, ICSI and IVF were performed according to standard procedures. Embryos were cultured in vitro at 37 °C in an atmosphere of 6 % CO_2 and 5 % O_2 in individual 25 μl droplets of sequential media formulations under paraffin oil. In total, 71 cycles were performed using Universal IVF Medium, EmbryoAssist[TM] and BlastAssist[®] (Medicult, De Pinte, Belgium), 357 cycles were performed using Vitrolife sequential media (G5 Series[TM], Vitrolife, Göteborg, Sweden) and 612 cycles were performed using Quinn's Advantage[®] protein plus sequential media (SAGE, Rochford Medical Ltd, Coventry, England). All systems were used according to manufacturer's instructions. Sixteen to 18 h post-insemination, fertilization was assessed by the presence of two pronuclei. On day 2 of preimplantation development, 4-cell stage human embryos developing from normally fertilized oocytes were examined once using inverted microscopy (magnification 400×) for inspection of the arrangement of the blastomeres within the embryo according to the previous cleavage planes. Hereby, tetrahedral-shaped 4-cell embryos were distinguished from non-tetrahedral-shaped embryos. Embryos that did not display a perfect tetrahedron and embryos displaying a non-tetrahedral arrangement were rolled in the culture dish in order to verify their arrangement. Fragmentation did not hinder the evaluation of blastomere arrangement. Annotations regarding blastomere arrangement were confined to two persons in order to limit inter-observer variability. These annotations were blinded for further embryo evaluations and embryo selection for transfer. Further development of the embryos was evaluated on a daily basis. Blastocyst evaluation relied on the scoring system described by Gardner and Schoolcraft [29]. Embryo transfer was performed on day 3 or day 5 using a soft catheter (K-Soft 5100, Cook, Bloomington, USA).

Outcome parameters

The embryological outcome parameters were embryo quality on day 3 and on day 5. Embryo quality on day 3 was based on cell stage and fragmentation rate. High-quality embryos had at least seven blastomeres and maximum 10 % of fragmentation. Good-quality embryos had at least seven blastomeres with more than 10 % but not more than 50 % of fragmentation or six blastomeres with maximum 20 % of fragmentation. Poor-quality embryos were defined as having more than 50 % of fragmentation, six blastomeres with more than 20 % of fragmentation or less than six blastomeres. Embryo quality on day 5 was based on blastulation stage and appearance of ICM and TE. High-quality embryos on day 5 were defined as blastocysts with at least a fully developed blastocoel (Gardner's Scale type 3 to 6) with a tightly packed ICM with many cells (Gardner's Scale A) and a good or average TE layer forming an epithelium with sufficient cells (Gardner's Scale A to B)

[30]. Good-quality embryos were defined as blastocysts with a loosely packed ICM (Gardner's Scale B) and a good or average TE (Gardner's Scale A to B) or as early blastocysts (Gardner's Scale type 1 to 2). Poor-quality blastocysts were defined as ICM/TE combinations in which at least one of both structures contained very few cells (Gardner's Scale C) or was absent (score D). Poor-quality embryos on day 5 included also embryos that failed to blastulate i.e. compacted embryos, cleavage stage embryos and degenerated embryos.

The clinical outcome measurements were positive βhCG, clinical pregnancy, and clinical pregnancy with fetal heart beat. A clinical pregnancy was defined as a pregnancy with an intra-uterine gestational sac observed at a transvaginal ultrasound scan performed at 7 weeks of gestation. A biochemical pregnancy was evidenced by the detection of βhCG without developing into a clinical pregnancy and with the exclusion of ectopic pregnancies. Definitions were adopted from Zegers-Hochschild et al. [31]. The clinical data were analysed for single embryo transfers (SETs) that were performed on either day 3 or day 5 and of which the transferred embryo was annotated as being a tetrahedral or a non-tetrahedral 4-cell stage embryo on day 2. SETs using embryos that did not expose four cells on day 2 and double embryo transfers were not considered for analysis.

Statistical analyses

The Chi-square test with Yates' correction was used to analyze the embryological data. The clinical data and the quality of the embryos transferred were analysed with the two-tailed Fisher's exact test. Female age, a predictive parameter for pregnancy, was compared between both groups with the independent Student's t-test. All tests were interpreted with a significance level of 95 % ($P < 0.05$).

Results

In total, 1,200 cycles were evaluated on day 2 of preimplantation development. In 1,040 cycles, at least one normally fertilized 4-cell stage embryo was present. In total, 3,546 4-cell stage embryos were examined for their arrangement of the blastomeres. Of these, 2,803 embryos (79 %) displayed a tetrahedral (T) arrangement, 743 embryos (21 %) displayed a non-tetrahedral (nT) arrangement. There was no significant difference in the percentage of T and nT embryos that originated from the different stimulation protocols (Chi-square, $p > 0.05$). 9.2 % of T embryos were created by conventional IVF compared to 12.5 % of the nT embryos (Chi-square, $p = 0.01$). There was no difference between the T and nT group in the percentage of embryos created with ejaculated sperm (92 % vs 91.8 %, respectively) and testicular sperm (8 % vs 8.2 %, respectively) (Chi-square, $p > 0.05$).

Embryological data

The embryological data are summarized in Tables 1 and 2. Embryo quality on day 3 was examined for 2,792 former T-shaped embryos and 742 former nT-shaped embryos (Table 1). T-shaped 4-cell stage embryos gave rise to significantly more high-quality embryos on day 3 ($p < 0.001$). nT-shaped embryos developed significantly more into poor-quality embryos ($p < 0.001$). The developmental potential towards the blastocyst stage on day 5 was available for 1,418 embryos, i.e. 1,147 T- and 271 nT-shaped 4-cell stage embryos (Table 2). Significantly more former T-shaped embryos developed into high-quality blastocysts ($p = 0.036$). nT-shaped embryos developed significantly more into poor-quality embryos on day 5 ($p = 0.002$). Poor embryo quality on day 5 included blastocysts with poor ICM and/or TE as well as non-blastulated embryos (compacted, cleavage stage and degenerated embryos). A Significant difference was found between T- and nT-shaped embryos regarding blastulation. T-shaped embryos had a higher chance to blastulate ($p = 0.009$). nT-shaped embryos arrested more at the cleavage stages ($p < 0.001$). The maternal age on day 3 and day 5 was not significantly different between the T and the nT embryos in the different quality groups ($p > 0.05$).

Clinical data

In total, 330 SETs were performed on day 3 and on day 5 using embryos that exposed four cells on day 2. The results are summarized in Tables 3 and 4. For day 3 as well as for day 5 embryo transfers, the female age was not significantly

Table 1 Embryo development on day 3

		T (n=2,792)		nT (n=742)		P-value
High-quality	≥7c F ≤10 %	1,801	64,5 %	398	53,6 %	<0,001
Good-quality	≥7c F >10-≤50 % or 6c F ≤20 %	770	27,6 %	217	29,3 %	0,394
Poor-quality	≥7c F >50 % or 6c F >20 % or <6c	221	7,9 %	127	17,1 %	<0,001

The table shows the quality of the transferred and non-transferred embryos on day 3

T tetrahedral, nT non-tetrahedral, n number, c cell stage, F fragmentation

Statistics: Chi square test with Yates' correction

Table 2 Embryo development on day 5

		T (*n*=1,147)		nT (*n*=271)		*P*-value
High-quality	At least full BL, ICM grade A, TE grade A or B	222	19,4 %	37	13,7 %	0,036
Good-quality	Early BL or BL with ICM grade B, TE grade A or B	406	35,4 %	83	30,6 %	0,157
Poor-quality	ICM and/or TE grade C or D	519	45,2 %	151	55,7 %	0,002
	Compacted, cleavage stage or degenerated embryos					
Blastulation	At least early blastocyst	812	70,8 %	169	62,4 %	0,009
No blastulation	Compacted embryos	238	20,7 %	62	22,9 %	0,491
	Cleavage stage embryos	66	5,8 %	35	12,9 %	0,001
	Degenerated embryos	31	2,7 %	5	1,8 %	0,554

The table shows the quality of the transferred and non-transferred embryos on day 5

T tetrahedral, *nT* non-tetrahedral, *n* number, *BL* blastocyst, *ICM* inner cell mass, *TE* trophectoderm

Blastocyst scoring according to Gardner and Schoolcraft (1999). Statistics: Chi square test with Yates' correction

different between the two groups. Out of the 195 SETs on day 3, 157 (81 %) came from the T group and 38 (19 %) from the nT group. On day 5, 135 SETs were performed, of which 111 (82 %) came from the T group and 24 (18 %) from the nT group. Pregnancy rates at the level of positive βhCG, biochemical pregnancy, clinical pregnancy and clinical pregnancy with fetal heart beat were comparable between the two groups for embryo transfers on day 3 and on day 5.

The quality of the embryos selected for SET on day 3 and on day 5 is summarized in Table 5. In the T group, 72.6 % of the embryos selected for SET on day 3 were of high-quality. In the nT group this was 60.5 % (*p*=0.167). More poor-quality embryos have been transferred on day 3 in the nT group (*p*= 0.024). On day 5, 70.3 % of the embryos from the T group were of high-quality versus 70.8 % of the embryos from the nT group (*p*~1). Cleavage stage embryos and degenerated embryos were not transferred on day 5.

Discussion

About one out of five (21 %) 4-cell stage human embryos did not display the expected tetrahedral arrangement of the blastomeres on day 2 of preimplantation development. This percentage of aberrant arrangement is similar to the proportions observed in mice [4, 32].

Our results showed significant differences in the developmental potential of tetrahedral- and non-tetrahedral-shaped 4-cell stage human embryos. Tetrahedral-shaped embryos had a higher chance to develop into high-quality embryos on day 3 and on day 5. In particular, non-tetrahedral-shaped embryos showed a reduced chance to form blastocysts and an increased risk to arrest at the cleavage stages. This finding is in line with Ebner et al. (2008) who observed that an ovoid zona pellucida (ZP) favors the generation of atypical cleavage patterns resulting in aberrant blastomere arrangements with a reduced number of contact points between them [33]. The low number of contact points was associated with delayed compaction and blastocyst formation. In our study, the examined embryos originated in general from round non-ovoid oocytes and ZP and so, no correlation could be made between the cleavage pattern and oocyte or ZP shape. The number of contact points between the blastomeres in our non-tetrahedral-shaped embryos was four. In the tetrahedral group, the number of contact points varied from four to six. Compaction normally occurs during the fourth cleavage division [34] and is mediated through E-cadherin [35, 36]. Cleavage abnormalities or loss of physical contact between blastomeres at an early

Table 3 Clinical outcome per single embryo transfer on day 3

	T (*n*=157)		nT (*n*=38)		*P*-value
Female age	33,5±5,14		34,3±5,07		0,349
Positive βhCG	45	28,7 %	7	18,4 %	0,226
Biochemical pregnancy	3	1,9 %	1	2,6 %	0,583
Clinical pregnancy	42	26,8 %	6	15,8 %	0,209
Clinical pregnancy with fetal heart beat	36	22,9 %	5	13,2 %	0,267

T tetrahedral, *nT* non-tetrahedral, *n* number

Statistics: Age: independent Student's *t*-test, Clinical outcome: two-tailed Fisher's exact test

Table 4 Clinical outcome per single embryo transfer on day 5

	T (n=111)		nT (n=24)		P-value
Female age	31±3,9		30,5±4,19		0,529
Positive βhCG	60	54,1 %	15	62,5 %	0,503
Biochemical pregnancy	3	2,7 %	1	4,2 %	0,547
Clinical pregnancy	57	51,4 %	14	58,3 %	0,653
Clinical pregnancy with fetal heart beat	53	47,8 %	13	54,2 %	0,655

T tetrahedral, nT non-tetrahedral, n number

Statistics: Age: independent Student's t-test, Clinical outcome: two-tailed Fisher's exact test

developmental stage can lead to disturbances in the distribution of E-cadherin which may induce compaction failure [37]. Nevertheless, neither the mean contact surface nor the number of contact surfaces of a day 3 embryo seems to have an additional value in embryo viability when performing day 3 transfer [38]. No correlation has been found between the "roundness" of a day 3 embryo and live birth following day 3 SET [39].

The observed developmental differences between the tetrahedral and non-tetrahedral embryos were not correlated to the different stimulation protocols. Contradictory to the results of Ebner et al. 2012, we did not find more non-tetrahedral embryos after ICSI and after testicular sperm extraction [40]. Hence, we could not confirm the assumption that the non-tetrahedral shape is under paternal influence. Authors also postulated that the non-tetrahedral arrangement could be the result of a mitotic spindle defect which in turn may cause aneuploidy [40]. Despite the small sample size and the use of arrested embryos that are known to carry a higher rate of aneuploidy [41], it might be an interesting path to explore.

In the present study, embryo selection for transfer was based on classical embryo morphology parameters. The tetrahedral or non-tetrahedral status of the embryo was blinded. Pregnancy rates on day 3 and on day 5 seemed independent of the tetrahedral/non-tetrahedral status on day 2. The percentage of high- and good-quality embryos selected for SET on day 3 and on day 5 was comparable. Though not significant, it is noteworthy that there was a benefit for the tetrahedral group of 11 % in terms of clinical pregnancy after SET on day 3. It can be hypothesized that compaction might also be a bottleneck for the non-tetrahedral embryos in vivo. However, once the former non-tetrahedral embryo went through the process of compaction and developed into a blastocyst of adequate quality on day 5, this embryo had equal chances for success compared to a former tetrahedral embryo.

Tetrahedral and non-tetrahedral embryos originate from differently orientated cleavage planes. Hereby, the polarized ooplasm may be distributed in another way to the blastomeres of tetrahedral and non-tetrahedral 4-cell stage embryos [5, 8, 11]. Although at a lower level, non-tetrahedral-shaped embryos were able to form high-quality blastocysts and transfer of such blastocysts led to normal pregnancy rates. Hence, the assumed altered distribution of ooplasm in non-tetrahedral-shaped embryos did not seem to have a clear effect on the formation of ICM and TE or on pregnancy outcome. The question whether early blastomeres are predestined to become either ICM or TE because of polarization in the oocyte has been challenged by Van de Velde et al. [42]. The four dissociated blastomeres of a tetrahedral-shaped 4-cell stage human embryo were individually capable to develop into blastocysts with ICM and TE. Therefore, the four blastomeres are equivalent regarding developmental potential. Whether the four blastomeres are also equivalent within the structure of the non-manipulated embryo is not known. However, randomly

Table 5 Quality of the embryos selected for single embryo transfer

Day 3 transfer		T (n=157)		nT (n=38)		P-value
High-quality	≥7c F ≤10 %	114	72,6 %	23	60,5 %	0,167
Good-quality	≥7c F >10-≤50 % or 6c F ≤20 %	42	26,8 %	12	31,6 %	0,550
Poor-quality	≥7c F >50 % or 6c F >20 % or <6c	1	0,6 %	3	7,9 %	0,024
Day 5 transfer		T (n=111)		nT (n=24)		
High-quality	At least full BL, ICM grade A, TE grade A or B	78	70,3 %	17	70,8 %	1,000
Good-quality	Early BL or BL with ICM grade B, TE grade A or B	25	22,5 %	5	20,8 %	1,000
Poor-quality	Compacted embryos or BL with ICM and/or TE grade C	8	7,2 %	2	8,4 %	1,000

T tetrahedral, nT non-tetrahedral, n number, c cell stage, F fragmentation, BL blastocyst, ICM inner cell mass, TE trophectoderm

Blastocyst scoring according to Gardner and Schoolcraft (1999). Statistics: two-tailed Fisher's exact test

labeling one out of four blastomeres showed that within the structure of the embryo this blastomere participates in both cell types of the blastocyst, i.e. ICM and TE [43]. Some murine studies showed that, depending on the orientation of the second cleavage planes, the four blastomeres expose different developmental capabilities [32, 44] and different epigenetic modifications [45, 46]. In tetrahedral-shaped embryos, the blastomeres have predictable fates. One of the blastomeres of the former 2-cell stage embryo is directed predominantly to the embryonic part and the other one to the abembryonic part of the blastocyst. When the orientation of the earlier of the second cleavage divisions is meridional, the polarity of this embryonic–abembryonic axis can be predicted [32]. In this formation, the blastomere that inherits only vegetal components contributes more to the mural TE. This blastomere also exposes decreased levels of methylation of arginine 17 and 26 on histone H3 [32, 45]. On the contrary, in non-tetrahedral-shaped embryos, no tendency for allocation was observed to specific parts of the blastocyst and no epigenetic variations were found [32, 44, 45]. Other studies in mice suggested no prelocalization of developmental determinants in the early blastomeres and showed that the second polar body is not a reliable morphogenetic determinant for the first cleavage [47, 48]. Some claim that there is no rotation of the spindle itself but a movement of the blastomeres [49].

From our study, it can be concluded that a non-tetrahedral arrangement of the blastomeres within the 4-cell stage human embryo may affect its subsequent development. These embryos fail more often to compact compared to their tetrahedral counterparts. This may be due to the reduced number of contact points between the blastomeres in a non-tetrahedral formation. Still, non-tetrahedral-shaped embryos are capable to form blastocysts with ICM and TE. Therefore, we do not expect a predisposition of the blastomeres that is linked to the inherited ooplasm. If the non-tetrahedral-shaped 4-cell stage embryos develop into adequate embryos at the moment of transfer, their pregnancy rates do not differ from embryos originating from a tetrahedral arrangement. Therefore, the present data do not support a benefit for the tetrahedral arrangement of blastomeres as a new selection criterion when the transfers are performed on day 3 and especially on day 5.

Limitations of this study are its retrospective and non-randomized design. Also, we could not distinguish between embryos that came from two meridional divisions and from two equatorial divisions within the non-tetrahedral group. The two types of non-tetrahedral embryos should be distinguishable from each other by the location of the second polar body [4, 32]. However, in practice, this is difficult because of polar body movement, polar body fragmentation and/or cellular fragmentation. Culturing in a time-lapse system could allow a more in dept analysis of the blastomere arrangement and could record possible movements or rotations of the blastomeres. It might be interesting to conduct a randomized controlled trial with transfer on day 2 after time-lapse culture in order to study the clinical significance of the blastomere arrangement at the 4-cell stage or the orientation of the second cleavage planes.

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