Clinical significance of intercellular contact at the four-cell stage of human embryos, and the use of abnormal cleavage patterns to identify embryos with low implantation potential: a time-lapse study

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Objective: To investigate the clinical significance of intercellular contact point (ICCP) in four-cell stage human embryos and the effectiveness of morphology and abnormal cleavage patterns in identifying embryos with low implantation potential.
Design: Retrospective cohort study.
Setting: Private IVF center.
Patient(s): A total of 223 consecutive IVF and intracytoplasmic sperm injection treatment cycles, with all resulting embryos cultured in the Embryoscope, and a subset of 207 cycles analyzed for ICCP number where good-quality four-cell embryos were available on day 2 (n = 373 IVF and n = 392 intracytoplasmic sperm injection embryos).
Intervention(s): None.
Main Outcome Measure(s): Morphologic score on day 3, embryo morphokinetic parameters, incidence of abnormal biological events, and known implantation results.
Result(s): Of 765 good-quality four-cell embryos, 89 (11.6%) failed to achieve six ICCPs; 166 of 765 (21.7%) initially had fewer than six ICCPs but were able to establish six ICCPs before subsequent division. Embryos with fewer than six ICCPs at the end of four-cell stage had a lower implantation rate (5.0% vs. 38.5%), with lower embryology performance in both conventional and morphokinetic assessments, compared with embryos achieving six ICCPs by the end of four-cell stage. Deselecting embryos with poor morphology, direct cleavage, reverse cleavage, and fewer than six ICCPs at the four-cell stage led to a significantly improved implantation rate (33.6% vs. 22.4%).
Conclusion(s): Embryos with fewer than six ICCPs at the end of the four-cell stage show compromised subsequent development and reduced implantation potential. Deselection of embryos with poor morphology and abnormal cleavage revealed via time-lapse imaging could provide the basis of a qualitative algorithm for embryo selection. (Fertil Steril® 2015;103:1485–91. ©2015 by American Society for Reproductive Medicine.)
Key Words: Embryo, time lapse, intercellular contact, in vitro fertilization, abnormal cleavage

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Since the introduction of time-lapse technology into human IVF programs, there has been an increasing volume of evidence showing improved pregnancy results via uninterrupted culture and continuous monitoring of human embryos

(1–3). However, recent debate has focused on whether the benefits can be attributed to optimized incubation conditions, improved embryo selection based on morphokinetic timings, or a combination of the two (4). Selection of embryos with high implantation potential by morphokinetic analysis invariably relies heavily on the timing of cell divisions (3, 5). Unfortunately, these quantitative measures can be affected by treatment protocols (6, 7), patient population (8), and technical aspects of the culture (9–11); algorithms developed using timings in one laboratory often lose specificity and sensitivity when transferred to another (12–14). To counteract this, the use of relative timing expressions rather than absolute timings to predict implantation of embryos has been proposed, potentially reducing inter-laboratory inconsistencies in embryo growth rates (15, 16). Another approach is to identify embryos with low implantation potential so they may be excluded or ranked lowly at the time of ET. A number of cleavage patterns have been identified associated with poor implantation rates, such as direct cleavage (DC) and reverse cleavage (RC) (17–19). These qualitative measures are relatively easy to identify using time-lapse systems, being ideal to confirm whether the anomaly occurred at any time during the culture period rather than just at the time of a static observation.

Intercellular communication is thought to be crucial to the development of preimplantation embryos (20–22). Because four-cell stage embryos are common at day-2 checks in human IVF laboratories, the assessment of the spatial arrangement of the four blastomeres (23–25) or the theoretically resulting number of intercellular contact points (ICCPs) (22) seems practical. The development and implantation potential of four-cell stage human embryos with tetrahedral or nontetrahedral (or planar) spatial configuration was recently reported by three separate embryology teams using static observations of embryos (23–25). However, the conclusions were conflicting, and it seems the definitions of spatial arrangement are unclear (26). Embryo development is a dynamic process, and therefore the status of embryo appearance can change with time (27), which can rarely be detected via static snapshot observations. The present study therefore focused on the number of ICCPs at the four-cell stage, as described in an earlier study (22), which seems more objective to define and easier to quantify.

The present study, with the assistance of continuous observations via time-lapse equipment, aimed to investigate [1] the prevalence and dynamic change of ICCP number in good-quality four-cell stage human embryos unimpeded by the presence of fragments, [2] the morphokinetics and implantation results of such embryos, and [3] the improvement of implantation likelihood based on a time-lapse deselection model incorporating a number of abnormal cleavage patterns.

MATERIALS AND METHODS

Ethics and Patient Selection

This retrospective study included a total of 193 women (aged 34.9 ± 4.5 years) (mean ± SD) with 223 consecutive IVF/intracytoplasmic sperm injection (ICSI) treatment cycles performed at Fertility North between February 2013 and September 2014. Informed consent was obtained from both partners of participating couples for the Embryoscope (Unisense; Fertilitech) incubation of embryos 3 days after oocyte collection. Retrospective data analysis was approved by the Research Ethics Committees at both Edith Cowan University and Joondalup Health Campus.

In 223 cycles, 207 (92.8%) were included for ICCP analysis at the four-cell stage, with at least one good-quality embryo available on day 2. Only good-quality embryos were included for ICCP analysis because large amounts of fragmentation can impede intercellular contact, making analysis difficult and potentially unreliable. A good-quality day-2 embryo was defined as [1] reaching the four-cell stage by 46 hours post insemination (hpi); [2] <20% fragmentation per volume of the embryo; and [3] showing no DC where zygotes underwent tripolar division resulting in three daughter cells or the two-cell stage was <5 hours (19), or one of the two-cell blastomeres underwent tripolar division resulting in three daughter cells or the four-cell stage was <5 hours. Using these criteria, 765 good-quality four-cell stage embryos (373 IVF and 392 ICSI) were analyzed for ICCP.

Patient Management and Embryo Culture

Controlled ovarian hyperstimulation, gamete preparation, and insemination via conventional IVF or ICSI were performed as previously described (18). Fertilized oocytes (16–18 hpi) were transferred to wells containing 25 μL ISM1 medium (Origio), each batch being supplemented with between 1.95 and 7.80 mg/mL human serum albumin (as per the manufacturer's specifications) and cultured for 3 days in the Embryoscope, and thereafter embryos were selected for uterine transfer and/or cryopreservation. Culture conditions in the Embryoscope were set at 6% CO_2, 5% O_2, and balanced N_2 at 37°C to maintain a pH between 7.2 and 7.4 in the ISM1 medium. Images were acquired in seven focal planes for each embryo every 10 minutes.

Annotation and Assessment of Embryo Development

Timing parameters documenting biological events for embryos were recorded for 3 days of culture using the Embryoviewer (Unisense; Fertilitech) software. Pronuclear fading (PNF) was used as the reference starting point to document cleavage timings against, rather than the time of insemination; so that inconsistencies between IVF and ICSI embryos may be removed as previously published (28). Relative timing expressions t4_t5 (duration of four-cell stage), cc2 (duration of two-cell stage), s2 (duration of 3-cell stage), and s3 (time from five- to eight-cell stage) were also recorded, together with abnormal biological events: multinucleation (MN) at two- and four-cell stages, DC as described earlier, and RC where two daughter blastomeres fused after complete separation (type 1) or failed cytokinesis after karyokinesis (type 2) (18). Finally, the number of ICCPs at the four-cell stage was documented, taking into account any changes over time, as illustrated in Supplemental Figure 1 (available online).

Conventional morphologic grading was performed as previously described (29), based on the embryo image captured by the Embryoscope at 68 hpi. Embryos were classified into four

grades between 1 (best quality) and 4 (poorest quality), taking into account cell number, symmetry/asymmetry of cells, and degree of fragmentation. Embryos scoring a grade 4 were defined as poor-quality embryos (PQEs). All annotations in the Embryoviewer software and conventional morphologic grading of embryos were performed by the same operator with more than 10 years' experience in clinical embryology. All embryologists (including the one mentioned above) in the laboratory performing the present study have been enrolled in the External Quality Assurance Schemes for Reproductive Medicine program (Northlands, Western Australia, Australia), including embryo grading and time-lapse monitoring schemes. Between-operator and between-laboratory variability is analyzed on a quarterly basis. Retraining is carried out should any operator's results fall above/below 5% of average overall results, so that consistency is maximized in all embryology analysis.

ET and Known Implantation Data

Either one or two embryos per patient were selected for transfer at the end of culture (3 days after oocyte collection), determined by the female patient's age and clinician's treatment plan. From the entire cohort of cycles (n = 223), a total of 196 treatment cycles resulted in ET, with the remaining 27 cycles having all embryos frozen owing to ovarian hyperstimulation syndrome risk or an elevated P level on the day of ovulatory trigger. Positive known implantation data (KID) was confirmed when the number of fetal hearts detected via ultrasound at 7 weeks of pregnancy matched the number of embryos transferred. Negative KID refers to embryos transferred in cycles with negative blood test results (β-hCG <25 IU/L) at 14 days after oocyte collection.

Statistical Analysis

Proportion parameters were compared using the Fisher exact test. Continuous data were analyzed via one-way analysis of variance followed by a least significance difference post hoc test. Relationships between continuous and/or categorical parameters were evaluated using Pearson's correlation or receiver operating characteristic (ROC) with area under the ROC curve test. Strength of association of different contributing variables with KID result was analyzed using multiple logistic regression. All statistical analysis was performed using Statistical Package for the Social Sciences 20.0 (SPSS), where $P<.05$ was considered statistically significant.

RESULTS

Cycle Characteristics

In the 207 IVF/ICSI cycles included for ICCP analysis (Table 1), a total of 70 cycles (33.8%) included at least one four-cell stage embryo with fewer than six ICCPs (type "A" or "B" in Supplemental Fig. 1) for the entire observed period, whereas 69 cycles (33.3%) included embryos where all had six ICCPs (type "C" in Supplemental Fig. 1) for the entire observed period during the four-cell stage. The remaining 68 cycles (32.9%) had at least one embryo showing fewer than six ICCPs, with all these embryos achieving six ICCPs by the end of the four-cell stage. Cycles in which all embryos had six ICCPs had significantly lower E_2 concentrations measured on the ovulatory trigger day (6,217.2 ± 3,661.1 pmol/L), compared with cycles in which at least one embryo had fewer than six ICCPs, irrespective of whether six ICCPs were eventually achieved (8,024.1 ± 4,373.4 pmol/L, $P<.05$) or not (7,682.8 ± 4,963.8 pmol/L, $P<.05$) (Table 1). Furthermore, cycles in which all embryos had six ICCPs also had a smaller number of oocytes collected compared with those cycles in which at least one embryo had fewer than six ICCPs at the end of the four-cell stage (9.1 ± 5.1 vs. 11.2 ± 6.7, $P<.05$) (Table 1). However, cycles with at least one embryo with fewer than six ICCPs at the end of the four-cell stage were not associated with female age, ovarian stimulating protocol, and duration of stimulation ($P>.05$, respectively; Table 1).

ICCP Prevalence in Good-quality Four-cell Stage Embryos

Table 2 shows the prevalence of ICCPs in 765 good-quality four-cell stage embryos according to female age and insemination methods. There was no difference in ICCP, in any

TABLE 1

Comparison of female stimulation cycle characteristics according to the number of ICCPs in four-cell stage embryos in 207 IVF/ICSI cycles for good-quality day-2 embryos only.

Variable	One or more embryo(s) with fewer than six ICCPs at the end of four-cell stage (n = 70, 33.8%)	Cycles involving: One or more embryo(s) starting with fewer than six ICCPs and all embryos achieving six ICCPs at the end of four-cell stage (n = 68, 32.9%)	All embryos showing six ICCPs throughout four-cell stage (n = 69, 33.3%)
Age (y)	35.0 ± 4.1	34.5 ± 5.1	35.6 ± 4.3
Stimulating protocol			
Agonist	23 (32.9)	15 (22.1)	20 (29.0)
Antagonist	47 (67.1)	53 (77.9)	49 (71.0)
Days of stimulation	10.8 ± 1.9	10.5 ± 1.8	10.3 ± 1.7
Peak E_2 (pmol/L)	7,682.8 ± 4,963.8[a]	8,024.1 ± 4,373.4[b]	6,217.2 ± 3,661.1[a,b]
No. of oocytes collected	11.2 ± 6.7[a]	10.1 ± 5.3	9.1 ± 5.1[a]

Note: Values are mean ± SD or number (percentage).
[a,b] Same superscript in the same row indicates statistical significance ($P<.05$).

Liu. Intercellular contact in 4-cell embryos. Fertil Steril 2015.

TABLE 2

Prevalence of ICCPs in 765 four-cell stage embryos according to female age and insemination type, for good-quality day-2 embryos only.

No. of ICCPs	No. of good-quality four-cell embryos (%)				
	IVF		ICSI		
	< 35 y	≥35 y	< 35 y	≥35 y	Total
Fewer than six	24 (10.9)	24 (15.8)	23 (11.3)	18 (9.6)	89 (11.6)
Achieves six	50 (22.6)	34 (22.4)	40 (19.6)	42 (22.3)	166 (21.7)
Six	147 (66.5)	94 (61.8)	141 (69.1)	128 (68.1)	510 (66.7)
Total	221 (100)	152 (100)	204 (100)	188 (100)	765 (100)

Note: Embryos were categorized as [1] fewer than six (i.e., four or five) ICCPs; [2] starting with fewer than six ICCPs but achieving six ICCPs by the end of the four-cell stage; or [3] six ICCPs for the entire observed period of the four-cell stage. No significant differences were detected between either of the two age groups or two insemination types ($P>.05$, respectively).

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category, between the two age groups (<35 or ≥35 years) or insemination types (IVF or ICSI). A total of 510 of 765 four-cell embryos (66.7%) had six ICCPs (type "C" in Supplemental Fig. 1) for the entire observed period of the four-cell stage, whereas 166 embryos (21.7%) had four (type "A") or five ICCPs (type "B" in Supplemental Fig. 1) at the beginning of the four-cell stage but achieved six ICCPs by the end. Furthermore, 166 of 255 (65.1%) of the four-cell embryos starting with fewer than six ICCPs (166 + 89) were seen to progress to six ICCPs by the end of the four-cell stage.

Development and Implantation of Good-quality Four-cell Embryos according to ICCP Status

Table 3 shows the development and implantation results of good-quality four-cell stage embryos according to their ICCP status. No significant differences were seen between embryos with different ICCP statuses in the incidence of abnormal biological events identified via time-lapse monitoring, including MN at two- ($P>.05$) or four-cell stage ($P>.05$) and RC before ($P>.05$) or after the four-cell stage ($P>.05$). However, embryos with fewer than six ICCPs at the end of the four-cell stage had significantly longer s2 (0.9 ± 1.5 vs. 0.6 ± 0.6, $P<.05$) compared with those achieving six ICCPs by the end of the four-cell stage, and they had significantly longer s3 (5.7 ± 4.8 vs. 4.1 ± 4.1, $P<.05$) than embryos with six ICCPs throughout the entire observed period of four-cell stage. Furthermore, embryos with fewer than six ICCPs at the end of the four-cell stage resulted in a significantly lower proportion of eight-cell embryos at 68 hpi when compared with either embryos achieving six ICCPs or embryos with six ICCPs throughout (52.8% vs. 70.5%, 52.8% vs. 70.0%; $P<.01$, respectively). In addition, embryos with fewer than six ICCPs at the end of the four-cell stage had larger (worse) conventional grading scores (1.9 ± 0.9 vs. 1.6 ± 0.8, 1.9 ± 0.9 vs. 1.6 ± 0.8; $P<.01$, respectively) and reduced KID implantation rate after ET (5.0% vs. 38.5%, $P<.01$; 5.0% vs. 27.0%, $P<.05$) compared with those achieving or having six ICCPs. Embryos that had six ICCPs at the end of the four-cell stage, regardless of whether they achieved it by the end of the four-cell stage or it was present throughout, resulted in a similar proportion of eight-cell embryos at 68 hpi ($P>.05$), conventional grading ($P>.05$), and KID implantation after ET ($P>.05$). Addtionally, no significant differences were detected in tPNF_t5, cc2, t4_t5, and tPNF_t8 between three ICCP groups ($P>.05$, respectively).

Further multiple logistic regression analysis was performed to test the strength of association of contributing variables with KID result (positive or negative), with odds ratio (OR) and 95% confidence internal (CI) analyzed. These factors included s2 (hours), cell number at 68 hpi (five, six, seven, or

TABLE 3

Time-lapse and conventional morphology data for 765 good-quality four-cell stage embryos according to the number of ICCPs observed via the Embryoscope, plus the KID result for a subset of 185 transferred embryos.

Embryo status	No. of ICCPs		
	Fewer than six	Achieves six	Six
Total no. of four-cell stage embryos	89	166	510
No. of embryos with MN at two-cell stage	18 (20.2)	39 (23.5)	100 (19.6)
No. of embryos with MN at four-cell stage	9 (10.1)	17 (10.2)	45 (8.8)
No. of embryos with RC before four-cell stage	2 (2.2)	5 (3.0)	18 (3.5)
No. of embryos with RC post four-cell stage	17 (19.1)	30 (18.1)	89 (17.5)
tPNF_t5 (h)	26.5 ± 3.3	26.6 ± 3.2	26.6 ± 3.1
cc2 (h)	10.9 ± 1.2	11.0 ± 1.1	10.9 ± 1.1
s2 (h)	0.9 ± 1.5[a]	0.6 ± 0.6[a]	0.7 ± 1.0
t4_t5 (h)	11.9 ± 2.3	12.3 ± 2.3	12.2 ± 2.2
No. reaching eight-cell stage by 68 hpi	47 (52.8)[b,c]	117 (70.5)[b]	357 (70.0)[c]
tPNF_t8 (h)	30.7 ± 4.8	30.4 ± 4.5	30.1 ± 4.5
s3 (h)	5.7 ± 4.8[a]	4.7 ± 4.2	4.1 ± 4.1[a]
Morphology grade	1.9 ± 0.9[b,c]	1.6 ± 0.8[b]	1.6 ± 0.8[c]
KID implantation	1/20 (5.0)[a,b]	15/39 (38.5)[b]	34/126 (27.0)[a]

Note: Values are presented as number (percentage) or mean ± SD. Embryos were categorized as [1] fewer than six (i.e., four or five) ICCPs, [2] starting with fewer than six ICCPs but achieving six ICCPs by the end of the four-cell stage, or [3] six ICCPs for the entire observed period of the four-cell stage. tPNF_t5 = time from pronuclear fading to five-cell stage; cc2 = duration of two-cell stage; s2 = duration of three-cell stage; t4_t5 = duration of four-cell stage; tPNF_t8 = time from pronuclear fading to eight-cell stage; s3 = time from five-cell to eight-cell stage.
[a] Same superscript in the same row indicates statistical significance ($P<.05$).
[b,c] Same superscript in the same row indicates statistical significance ($P<.01$).

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eight), conventional morphology grade (1, 2, or 3) and never achieving six ICCPs ("<6 ICCP"; yes or no), but s3 was excluded to avoid embryos that did not reach eight-cell stage by 68 hpi from being removed from analysis. Results indicated that fewer than six ICCPs stood as a stronger factor (OR 0.128; CI 0.016–0.998; $P<.05$) affecting KID result over the other variables, including s2 (OR 0.596; CI 0.288–1.233; $P>.05$), cell number at 68 hpi (OR 1.862; CI 0.786–4.414; $P>.05$), and morphology grade (OR 0.783; CI 0.356–1.7233; $P>.05$).

Time Taken to Achieve Six ICCPs and Subsequent Development and Implantation

In the good-quality four-cell stage embryos that successfully achieved six ICCPs by the end of the four-cell stage (n = 166), Pearson correlation analysis was performed to investigate the relationship between the time taken to achieve six ICCPs during the four-cell stage and the embryo's subsequent development. No correlations were found between the time taken to achieve six ICCPs and the cell number (five, six, seven, or eight) at 68 hpi ($r = 0.018$, $P=.822$), conventional grade ($r = 0.072$, $P=.355$), t4_t5 ($r = 0.042$, $P=.595$) and s3 in those having reached eight-cell stage (n = 117, $r=-0.003$, $P=.970$). In a subset of 39 transferred embryos with KID result, a ROC test showed no predictive value on implantation by the time taken to achieve six ICCPs (area under the ROC curve = 0.451, $P=.613$).

Improvement of Implantation Rates via Time-lapse Deselection of Embryos

Table 4 shows KID for the 223 transferred embryos with an implantation rate of 22.4%. Embryos were sequentially deselected according to the qualitative criteria of PQE based on conventional grading at 68 hpi, DC, RC, and fewer than six ICCPs at the four-cell stage. Deselecting embryos for the listed criteria (i.e., removing them from the cohort) led to a significantly increased implantation rate of 33.6% ($P<.05$) with a power ($1-\beta$) of 0.92 when type 1 error rate (α) was set at 0.05. Indeed, from the 50 embryos that implanted, only 1 embryo was from the deselected criteria group, which only showed fewer than six ICCPs throughout the observations of the four-cell stage.

DISCUSSION

Evidence has shown that reduced intercellular communication due to the loss of intercellular contact in early cleavage-stage human embryos may result in compromised subsequent development (21, 22, 30). With the use of continuous time-lapse monitoring, the present study investigated in detail the prevalence and dynamic changes of the ICCP status of four-cell stage human embryos (Supplemental Fig. 1), together with their subsequent morphokinetic data and implantation outcome. Only good-quality four-cell embryos were included for analysis, and embryos with high levels of blastomere fragmentation that could potentially affect the reliability of the observations were excluded. Overall one-third of all analyzed embryos had fewer than six ICCPs at some point during the four-cell stage, similar to a previous study (22). Interestingly, nearly two-thirds (65.1%) of these embryos eventually corrected the suboptimal structure, establishing six ICCPs by the end of the four-cell stage. Such embryos, which eventually achieved six ICCPs, often displayed blastomeres that migrated toward each other, gradually establishing contact, as shown in Supplemental Videos 1 and 2. The identification of dynamic changes such as ICCP number is a benefit of time-lapse culture, which otherwise might not be achieved in conventional embryo culture using static observations. Furthermore, the embryos that eventually achieved six ICCPs had comparable implantation rates to embryos that showed six ICCPs throughout the entire observations (Table 3). Conversely, embryos that never achieved six ICCPs had a significantly lower implantation rate compared with those that did achieve six ICCPs, suggesting "<6 ICCP" at the end of the four-cell stage, observed using time-lapse monitoring, could be incorporated as a deselection criterion in clinical practice. It is worth noting that in static observations, embryos that eventually achieve six ICCPs may be categorized as either six ICCPs throughout or fewer than six ICCPs, depending on the phase of the four-cell stage at the time of observation, thus introducing a potentially confounding variable.

In line with the dynamic changeable nature of ICCP status at the four-cell stage, embryos categorized with an initial non-tetrahedral spatial arrangement can migrate into a tetrahedral configuration (Supplemental Video 1). Furthermore, not all tetrahedral four-cell embryos have six ICCPs; some have five ICCPs throughout the entire stage, which have been shown in this study to have compromised development and implantation potential (Table 3). This could explain the conflicting conclusions between the three previous studies (23–25), which focused on the tetrahedral vs. non-tetrahedral (or planar) configuration of the four-cell stage embryos. In addition, there are occasions where the cleavage axis of each blastomere in the two-cell stage embryo is not perfectly at 0° or 90° off the animal–vegetal axis, because some two- to four-cell divisions may occur along two axes at an intermediate angle (i.e., 30°–60°) (31). In such embryos one operator may categorize the four-cell embryos as tetrahedral but the other as non-tetrahedral. Therefore, the number of ICCPs at the four-cell stage, observed in time-lapse monitoring, may be a more objective measure to categorize blastomere contact compared with spatial arrangement descriptions. However, it is worth noting there were occasions during the time-lapse monitoring when the ICCP status could not be determined owing to a suboptimal viewing angle by the camera and the inability to roll the embryos.

TABLE 4

Implantation rates after the application of deselection criteria.

Embryo deselection criterion	No. of embryos transferred	No. of implanting embryos (%)
All embryos	223	50 (22.4)[a]
Less PQE	208	50 (24.0)[b]
Less PQE, DC	185	50 (27.0)
Less PQE, DC, and RC	167	50 (29.9)
Less PQE, DC, RC, and fewer than six ICCPs	146	49 (33.6)[a,b]

[a,b] Same superscript indicates statistical significance ($P<.05$).

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The occurrence of fewer than six ICCPs at the four-cell stage seemed to be associated with increased ovarian stimulation, as indicated by the number of oocytes collected and concentration of serum E_2, but not with female age, days or protocol of ovarian stimulation, and insemination methods (Tables 1 and 2). It has been reported that supraphysiologic E_2 levels are associated with reduced pregnancy rates (32), and the adverse outcome is not limited to the compromised endometrial receptivity only but may also affect embryo development viewed during static observations (33) or time-lapse videography (6). Because the number of oocytes collected is correlated with the serum E_2 concentration (32), it is not surprising that the results in the present study showed a similar pattern with the occurrence of fewer than six ICCPs being associated with an increased oocyte number.

Embryo deselection has routinely been used by embryologists to rank embryos for uterine transfer or cryopreservation in conventional grading systems, assessing the presence of fragmentation or unevenness of blastomeres via static observations (29). With the use of time-lapse incubators there is increased opportunity to observe abnormal biological events, enabling novel parameters of embryo quality to be considered (17–19, 34). The advantage of documenting deselection criteria such as DC, RC, and fewer than six ICCPs is they are qualitative measurements, independent of absolute cleavage timings of observed embryos. Such qualitative measurements may have improved transferability between different laboratories and culture systems, and could aid the development of a deselection algorithm; especially given that the reproducibility of positive selection of embryos based on cleavage timings has recently been questioned (12, 14). Again, such variability in timings is probably due to differences in culture conditions between different laboratories (9–11). The present study incorporated a number of abnormal cleavage patterns, including DC, RC, and fewer than six ICCPs, to establish a deselection model showing significant improvement in the implantation rate of embryos that did not display these abnormal cleavage events (Table 4). Because the model is independent of the absolute timings of biological events, as mentioned above, improved reproducibility between laboratories with different culture conditions may be expected; however, a multicenter study is required to confirm this.

A limitation of the present study is its retrospective nature, as well as the exclusion of poor-quality embryos from ICCP analysis, making it difficult to control for confounding variables in this design. Therefore future randomized, controlled, prospective studies are warranted to validate findings in this study. Larger multicentered studies must involve extensive training of embryologists to achieve improved consistency in the identification of abnormalities of cleavage, as has been done previously with conventional embryo grading (35, 36). Their effectiveness can be determined using intraclass correlation coefficients as shown in a previous publication, where the inter- and intraobserver variability when annotating embryos using the Embryoviewer was shown to be at a satisfactory level (37). The measuring tools in the Embryoviewer can also produce high intraobserver reproducibility (e.g., the "Ellipse" tool as shown in a previous publication by the present authors [38]).

In conclusion, the present study identified that [1] nearly two-thirds of embryos showing fewer than six ICCPs during the four-cell stage can achieve six ICCPs by the end of this stage; [2] embryos with fewer than six ICCPs at the end of the four-cell stage had compromised subsequent development and implantation; [3] occurrence of fewer than six ICCPs is independent of female age, protocol, or duration of ovarian stimulation, and insemination methods but is associated with larger number of oocytes collected and elevated peak E_2 levels; and [4] implantation rates could be improved by using a deselection algorithm based on morphology and abnormal cleavage patterns identified via time-lapse observations.

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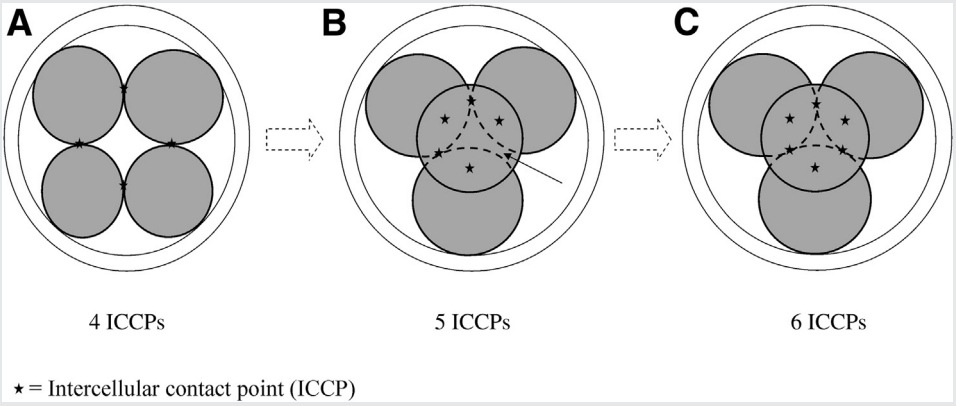
SUPPLEMENTAL FIGURE 1



Different types of four-cell stage human embryos showing the (**A-C**) number of intercellular contact points (ICCPs).

Liu. Intercellular contact in 4-cell embryos. Fertil Steril 2015.