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Short title: Prevalence and implantation potential of phenotypically abnormal embryos

Preliminary investigation of the prevalence and implantation potential of abnormal embryonic phenotypes assessed using time-lapse imaging

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Key message

Embryos with abnormal division patterns as revealed by time-lapse microscopy have reduced developmental capacity and implantation potential compared with their normal counterparts. These findings emphasize the utility of time-lapse technologies in the embryology laboratory.



Author Biography

Amy Barrie studied at Manchester University and University College London and currently holds a Masters of Science in Prenatal Genetics and Fetal Medicine. She began working as a Clinical Embryologist at the Hewitt Fertility Centre, Liverpool in 2009 and contributes to one of the largest NHS providers of fertility treatments in the UK. Amy is currently

undertaking a PhD investigating time-lapse imaging systems and how they can be used to improve the success of fertility treatment.

Abstract

This retrospective, single site observational study aimed to delineate five abnormal embryonic developmental phenotypes, assessing their prevalence, development potential and suitability for inclusion in embryo selection models for IVF. In total, 15,819 embryos from 4559 treatment cycles cultured in EmbryoScope® incubators between January 2014 and January 2016 were included. Time-lapse images were assessed retrospectively for five abnormal embryo phenotypes: direct cleavage, reverse cleavage, absent cleavage, chaotic cleavage and cell lysis. The prevalence of each abnormal phenotype was assessed. Final embryo disposition, embryo quality and implantation rate were determined and compared with a control embryo cohort. The collective prevalence for the five abnormal phenotypes was 11.4%; chaotic cleavage and direct cleavage together constituted 9.7%. Implantation rates were 17.4%, 0%, 25%, 2.1% and 0% for direct, reverse, absent, chaotic cleavage and cell lysis, respectively. The overall implantation rate for all abnormal embryos with known implantation status was significantly lower compared with the control population (6.9% versus 38.7%, $P < 0.0001$). The proportion of good quality embryos in each category of abnormal cleavage remained below 25%. Embryos exhibiting an abnormal phenotype may have reduced developmental capability, manifested in both embryo quality and implantation potential, when compared with embryos of normal phenotype.

Keywords: *abnormal phenotype, embryo development, morphokinetics, time-lapse*

Introduction

Abnormal cleavage patterns exhibited by some embryos include, but are not limited to: abnormal syngamy, direct cleavage (DC), reverse cleavage (RC), absent cleavage in the presence of karyokinesis (AC), chaotic cleavage (CC) and cell lysis (CL).

The first of five abnormal cleavage patterns investigated here is DC. This is the cleavage of one blastomere into three, instead of the expected two, daughter cells (**Supplementary Figure 1**). The ability of these embryos to establish a pregnancy has been shown to be significantly reduced: 13.7% of all examined embryos and 6.6% of transferred embryos underwent DC, with 1.2% resulting in a clinical pregnancy (Rubio *et al.*, 2012). These embryos have been shown to have a markedly decreased blastocyst formation rate when compared with their normal counterparts (Athayde Wirka *et al.*, 2014).

The second abnormal phenotype to be considered is RC, the phenomenon of blastomere fusion (**Supplementary Figure 1**). Of 789 embryos assessed for RC, defined as blastomere fusion or failed cleavage, 27.4% of embryos were found to exhibit this abnormal cleavage pattern and were

shown to have a reduced implantation potential (Liu *et al.*, 2014). An examination of 1698 embryos detected a prevalence of RC of 6.8%; however, embryos appeared to have similar fragmentation, cell evenness and morphokinetic profiles compared with their non-RC counterparts (Hickman *et al.*, 2012). This research concluded that RC does not seem to impair embryo development to the blastocyst stage, a finding supported by those of others (Desai *et al.*, 2014).

AC is defined as the process by which a blastomere undergoes a pseudo division (seen as a 'roll') that does not produce two discernable blastomeres but a single, or multiple, extra nuclei within the single blastomere (**Supplementary Figure 1**). AC has previously been categorized under RC, termed type II RC (Liu *et al.*, 2014). Of those embryos that underwent RC (27.4%), 82% were classified as type II: absent cleavage rather than blastomere fusion. Further evidence of this specific developmental pattern has not yet been published. This is perhaps due to the likelihood that these embryos will not be used for treatment, thus circumventing a clinical need to further define this phenomenon.

CC results when an embryo undergoes apparent cleavage but does not create distinctive blastomeres (**Supplementary Figure 1**). A single investigation studying this cleavage pattern in 639 embryos found an overall prevalence of 15%, a blastocyst formation rate of 14% and an implantation rate (IR) of 0% (Athayde Wirka *et al.*, 2014). Interestingly, this investigation also found that 35.2% of those exhibiting CC had good cleavage-stage quality. This was, however, markedly lower than the other abnormal phenotypes observed (DC and abnormal syngamy). Again, as with AC, this phenomenon may be under-investigated due to the reduced likelihood that embryos exhibiting this phenotype will be used in treatment.

Finally, an abnormal embryo developmental phenomenon that has yet to be discussed in the literature, in terms of time-lapse imaging of embryos from fresh treatment cycles, is CL (**Supplementary Figure 1**), a process often visualized in frozen-thawed embryos (Bottin *et al.*, 2015; Rienzi *et al.*, 2005; Tang *et al.*, 2006; Yeung *et al.*, 2009). In an analysis of 891 frozen embryo transfer (FET) cycles, no pregnancies resulted if CL occurred in over 50% of the embryos. However, if CL accounted for 25 to 50% of the embryos, the pregnancy rate was 3.2%, significantly lower than if less than 25% CL had occurred (16.6%) (Tang *et al.*, 2006). This finding is supported by others (Bottin *et al.*, 2015; Yeung *et al.*, 2009).

Although these investigations are not entirely synonymous with the current analysis, they provide evidence that embryos with lysed cells have a reduced implantation potential.

As discussed above, there is disparity in the literature regarding the prevalence and implication of the presence of certain abnormal phenotypes. Further investigation into these phenomena is required to determine if their presence is reason to exclude these embryos from selection for use in treatment. Five abnormal cleavage patterns exhibited by embryos (DC, RC, AC, CC and CL) are explored in 15,819 embryos,

detailing their prevalence, implantation potential, and the suitability for inclusion of these potential deselection criteria in embryo selection models.

Materials and methods

This investigation was a single site, retrospective observational design approved by the North West Research Ethics Committee (ref: 14/NW/1043) and the Institutional Review Board of Edge Hill University. All procedures and protocols complied with UK regulation (Human Fertilization and Embryology Act, 1990, 2008). Data were obtained from 4559 treatment cycles including 15,819 embryos cultured in the EmbryoScope® incubators between January 2014 and January 2016.

Ovarian stimulation

Pituitary down-regulation was achieved using either a gonadotrophin-releasing hormone (GnRH) agonist (buserelin, Suprecur®, Sanofi Aventis, UK) or antagonist (cetrorelix acetate, Cetrotide®, Merck Serono, Germany). Ovarian stimulation was performed using urine-derived or recombinant FSH (Progynova (Bayer, Germany), Fostimon, Merional (IBSA, Switzerland), Menopur® (Ferring Fertility, Switzerland), Gonal f® (Merck Serono). Doses were adjusted based on patient demographic and response. Patients were given 5000 IU of subcutaneous human chorionic gonadotrophin (HCG) (Gonasi® HP, IBSA Pharmaceuticals, Italy) 36 h prior to oocyte collection. Luteal support was provided using 400 mg of progesterone pessaries twice daily (Cyclogest®, Actavis, UK) until the pregnancy test was performed.

Oocyte retrieval and embryology

Ultrasound-guided oocyte collection was performed transvaginally under sedation (Diprivan, Fresenius Kabi, USA). Collected oocyte cumulus complexes were cultured in four-well dishes (Nunc™, Thermo Scientific, USA) each well containing 0.65ml GIVF™ (Vitrolife, Gothenburg, Sweden) covered with 0.35 ml OVOIL™ (Vitrolife) in a standard incubator (Sanyo Multigas MCO 18M). Sperm preparation was performed using a standard gradient separation (ISolate®, Irvine Scientific, USA) at 0.3 relative centrifugal force (rcf) for 10 min followed by two washes at 0.6 rcf for 10 min using GIVF™. Those oocytes destined for intracytoplasmic sperm injection (ICSI) were prepared using enzymatic (HYASE 10X™, Vitrolife) and mechanical digestion. ICSI was performed on all metaphase II (MII) oocytes approximately 4 h following collection, after which time all injected oocytes were placed in individual culture drops of G1™ (for all cycles before September 2014) or GTL™ (all cycles after September 2014) (Vitrolife) and cultured in the EmbryoScope® (Vitrolife). Those oocytes destined for standard insemination (IVF) had this performed approximately 4 h after collection and were replaced into a standard incubator until the following day. Oocytes were then checked for fertilization approximately 16 to 18 h post-insemination (hpi) and all fertilized oocytes along with all unfertilized MII oocytes were placed in individual culture drops as with ICSI derived embryos and cultured in the

EmbryoScope®. Embryo selection was performed using the national grading scheme (ACE/BFS guidelines (Cutting *et al.*, 2008, **Supplementary Table 3**)) along with an internally derived, embryo scoring algorithm (ESA). An ESA seeks to combine a number of morphokinetic parameters that have been linked to an embryo's viability. The ESA employed here was used as an additive to morphology, with the latter remaining the gold standard. This ESA included three morphokinetic parameters; s2 (time between t3 and t4), cc3 (time between t4 and t5) and t5 with embryos graded in one of eight categories from A+ to D- (**Supplementary Table 4**). Embryo transfer was performed using the highest grade embryo(s) either 3 or 5 days post-collection, depending on the number of GQE the patient had on day 3 as well as how many were to be transferred. Selected embryos were cultured in EmbryoGlue® (Vitrolife) for 10 to 30 min in a standard incubator prior to embryo transfer. Embryos were cultured at 37°C, 6% CO₂, 5% O₂, 89% N₂ throughout.

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Analysis of time lapse information

The image interval on the EmbryoScope® was set to 10 min with seven focal planes. Images were collected for the duration of culture immediately following ICSI or fertilization check (for IVF derived embryos) to utilization. Images were assessed by an embryologist for the abnormal embryonic phenotypes of interest. For DC, embryos were classified into one of three categories: true DC (TDC, defined as all three resultant cells cleaving on the subsequent cell cycle, each having a nucleus and each included in the morula); false DC (FDC, one or more of the above criteria not fulfilled); and unconfirmed DC (UDC, unable to classify as true or false). UDC embryos were defined as such due to either obscurity preventing categorization or the cessation of culture before the morula stage was reached. A justification for the choice of this classification, not reported elsewhere, lies in unit-specific data whereby two obviously distinct DC event patterns were visualized using time-lapse technology. This, as well as previous reports of direct cleavage patterns (Kalatova *et al.*, 2015; Kola *et al.*, 1987), led to the development of the three-tiered classification of DC events. Regarding the final criterion for TDC classification (inclusion of all cells in the morula), this stage of development was used as an indicator that all cells, abnormal or not, would contribute to the eventual blastocyst and would not be excluded. Further to this, DC could be proposed as a correction mechanism whereby the DC event is a means to remove surplus genetic material, thus excluding the cells from the eventual blastocyst, described here as FDC and a more favourable type of DC event. Direct cleavage from both one to three cells (DC1–3) and from two to five cells (DC2–5) were included in the analysis. RC is defined simply as blastomere fusion. AC is defined as the process by which a blastomere undergoes a pseudo division (seen as a 'roll') that does not produce two discernable blastomeres but a single, or multiple, extra nuclei within the single blastomere. CC is observed when an embryo undergoes apparent cleavage but does not create distinctive blastomeres. CL is defined as the loss of a blastomere through cell lysis (**Supplementary Figure 1**). Although not exclusively a phenomena visualized through time-lapse technology and one that can be visualized

using standard embryo morphology assessment, CL is predominantly seen in embryos following cryopreservation, whereas here we describe cell lysis in fresh embryos. Thus, this was included in the current investigation to determine the effect of cell lysis on the viability of a fresh embryo.

Outcome measures and statistical analysis

The overall prevalence of the five abnormal embryo phenotypes was defined per embryo and per treatment cycle. The average patient age, oocytes collected and previous attempts were calculated for each of the five categories. The fate (transfer, freeze, discard) of each abnormal embryo was determined as well as its quality on the day of utilization, defined as good, average or poor (**Supplementary Table 1**). The IR for each abnormal phenotype was determined. For this analysis, no transfers in which an abnormal embryo was transferred with a normal embryo were included and only those for which the origin of the fetal heart was confirmed were included, i.e. known implantation data, using single embryo transfers of an abnormal embryo or double embryo transfers where two abnormal embryos were transferred resulting in a negative outcome or two fetal heartbeats. The number of single and double abnormal embryo transfers and the stage at which the abnormal embryo(s) was transferred was also determined (**Supplementary Table 2**). Statistical analyses included Student's *t*-test for the comparison of the abnormal phenotype baseline information (patient age, oocytes collected and previous attempts) to the control embryo baseline data. The Fisher's exact test was used to compare the IR of the abnormal embryos with normal counterparts. Results were considered significant at $P < 0.05$. Statistical analysis was performed using the statistical package Prism® 5 (GraphPad Software©, USA).

Results

Data were obtained from 15,819 embryos from 4559 treatment cycles cultured in the EmbryoScope® between January 2014 and January 2016. Of the 15,819 embryos, 14,008 were derived from 3273 treatment cycles where no abnormal divisions of interest (DC, CC, RC, AC and CL) were observed and thus constituted the control group. The remaining embryos (1811) were found to pertain to a treatment cycle ($n = 1286$) exhibiting an embryo with one of the abnormal division patterns of interest.

Abnormal phenotypes with the highest prevalence per embryo observed were DC and CC at 4.4% (TDC, FDC, UDC, collectively) and 5.3%, respectively. The remaining phenotypes had considerably lower prevalence ranging from 0.41 to 0.8% (**Table 1**). The overall prevalence per embryo observed of abnormal division patterns was 11.4% (**Table 1**).

The embryos from the control group (not undergoing an abnormal division event; 14,008) resulted in 3456 embryos transferred and 1336 fetal heartbeats, giving an IR of 38.7% (**Table 1**). These transfers resulted in a clinical pregnancy rate (CPR) of 38.8% (presence of fetal heart(s) in 1269 of 3273 embryo transfers). The transfer of a total of 86 abnormal embryos resulting in 6 fetal heartbeats gave an overall IR of 7.0% (**Table 1**),

resulting in a CPR of 8.3% (presence of fetal heart(s) in 6 of 72 embryo transfers). Overall CPR for transfers including at least one abnormal embryo can be found in **Supplementary Table 5**. Of the five abnormal division patterns, the IR of UDC, CC and RC were significantly lower than normal counterparts: 12.5% ($P = 0.0378$), 2.1% ($P < 0.0001$) and 0% ($P = 0.0153$), respectively (**Table 1**). Furthermore, the overall IR of all abnormal embryos was significantly lower than normal counterparts (7.0% versus 38.7%, $P < 0.0001$) (**Table 1**) and of the six implanted abnormal embryos, five resulted in a live birth, with no birth defects, and one remains ongoing. In all cases the percentage of GQE resulting from those exhibiting abnormal division patterns never reached above 24% and the majority of embryos were classified as poor quality (**Table 1**). This is also reflected in the utilization of these embryos; the highest proportion of each group was discarded (**Supplementary Figure 2**). The number of embryos undergoing either DC1–3 or DC2–5, respectively, in each of the DC categories was as follows: TDC, 16 (2.3%) and 32 (4.6%); FDC, 26 (3.7%) and 43 (6.2%); UDC, 176 (25.3%) and 404 (58.0%).

Patient age was significantly lower for cycles with embryos exhibiting DC ($P < 0.0001$), RC (0.0097) and CC ($P < 0.0001$) compared with those not exhibiting an abnormal division pattern. The number of oocytes collected was found to be significantly higher in treatment cycles containing abnormal embryos than in those not containing embryos exhibiting an abnormal division pattern ($P < 0.0001$). Finally, the number of previous attempts was not found to be significantly different between any of the abnormal division categories and the control embryo cohort (**Table 2**).

Discussion

The prevalence of DC in the literature has been stated as 13.7% (Rubio *et al.*, 2012) and 18% (Hickman *et al.*, 2012). In the current analysis the overall prevalence of DC was 4.4% (UDC, FDC and TDC combined) occurring in 1.22 embryos per treatment cycle. The implantation potential of embryos undergoing DC has been stated as just 1.2% (Rubio *et al.*, 2012); however, in the current analysis the IR was found to be 17.4% (4/23; TDC, FDC and UDC combined), not significantly lower than that of the control embryo cohort. A classification system of DC was not adopted by other publications; therefore, if FDC were not considered, the IR would be significantly lower than those not exhibiting a DC. Of the three categories, those that were classed as FDC had the highest IR, as one might expect from the definition. There is a paucity of literature regarding the exact mechanisms underlying the phenomenon of DC; however, it has been speculated that the presence of a multipolar spindle could be a contributing factor (reviewed in Kalatova *et al.*, 2015). In addition, the presence of surplus centrosomes leading to DC is reflected in an early investigation of tri-pronucleate oocytes. Genetic assessment of tri-pronucleate DC oocytes revealed three division patterns: DC to three cells (62%); cleavage to a morphologically normal 2-cell 'embryo' (24%); and cleavage to a 2-cell 'embryo' plus an extrusion (14%) (Kola *et al.*, 1987). All tri-pronucleate oocytes that had undergone DC to three cells were chromosomally abnormal with each containing a varied number of chromosomes (here considered a TDC). Those that cleaved to

morphologically normal 2-cell 'embryos' were found to be true triploid with each blastomere containing a 69XXX/XXY chromosome complement. However, of those oocytes that cleaved to a 2-cell 'embryo' plus an extrusion, 75% were found to have two diploid blastomeres and a haploid extrusion. In the analysis presented here, of those embryos analogous to the 2-cell embryo plus an extrusion, two of six implanted. Caution should be taken as the numbers are small in this group due to the need to use known implantation embryos, however, this represents a result just over 5% lower than that of a phenotypically normal embryo. Although speculative, the findings by Kola *et al.* (1987) could also indicate that embryos have the potential to correct genetic abnormalities. There are many studies detailing self-correction between the cleavage stage and the blastocyst stage of embryo development (Barbash-Hazan *et al.*, 2008; Li *et al.*, 2005; Munne *et al.*, 2005; Northop *et al.*, 2010; Voullaire *et al.*, 2000). It has been noted that trisomic embryos correct more often than other aneuploidies (Barbash-Hazan *et al.*, 2008), possibly occurring through the loss of a chromosome in trisomic cells (Munne *et al.*, 2005). In addition, in previous reports, CC could be misinterpreted as a DC, thus causing the prevalence of DC to appear falsely increased. The increased IR of DC seen in the present investigation compared with previous reports may also be due to observers having experience with the different categorizations of DC, making them proficient at recognizing patterns of FDC, such as blastomere behaviour, allowing preferential selection of a potential FDC in UDC cases. The reduced patient age and increased number of oocytes collected may reflect a simple association between maternal age and number of oocytes collected. However, it may also indicate that stimulation can lead to reduced oocyte quality (Aboulghar *et al.*, 1997) and high oocyte numbers (>15) can reduce the chance of a live birth (Ji *et al.*, 2013), which could manifest as an abnormality such as DC.

RC occurred in 65 embryos (1.07 embryos per treatment cycle), of which 36 were either transferred or frozen, and 25 of those were classed as good or average quality. It is likely that embryos classed as poor quality embryos (PQE) were utilized due to the unavailability of others. None of the nine transferred embryos that underwent RC implanted in the current investigation. The prevalence of RC has been reported as 6.8, 7 and as high as 27.4% in previous reports (Desai *et al.*, 2014; Hickman *et al.*, 2012; Liu *et al.*, 2014). However, the rate of formation of usable embryos is in conjunction with others at approximately 40% (Desai *et al.*, 2014). There have been reports that RC is affected by other variables such as ICSI and GnRH antagonists. Therefore a possible explanation for the disagreement presented here could be due to the difference in baseline patient and treatment variables, a consideration for further investigation. The phenomenon of RC has been recognized previously with regards to frozen-thawed embryos (Balakier *et al.*, 2000; Trounson, 1984). Balakier *et al.* (2000) sought to determine the chromosomal changes in blastomeres that undergo fusion following thawing. RC was found in 51 of 2014 embryos of which 70% were classed as good quality. The overall frequency of RC was 4.6% in day 2 embryos and 1.5% in day 3 embryos. A slightly higher incidence of blastomere fusion was found in embryos created using IVF when compared with ICSI. When a control group was observed (embryos not subject to freezing and thawing) the prevalence of

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RC was 0.3%, a result not far from that recorded in the present study (0.4%). The IR of embryos that underwent blastomere fusion following thawing in the above investigation was very poor, with 15 embryo transfers containing one abnormal and one normal embryo resulting in only one live birth –again, a result similar to that seen in the present investigation. The chromosomal status of blastomeres resulting from fusion was also examined where embryos affected by RC were transformed into either polyploidy or mosaic embryos. The authors suggested that the occurrence of blastomere fusion could be associated with existing membrane abnormalities that could promote fusion affected by factors such as pH, temperature and osmolality differences. Interestingly, in some fields of research the production of tetraploid embryos is advantageous, and it has been concluded that tetraploidy does not prohibit preimplantation development (Eglitis, 1980), corroboration for the development of approximately 40% good/average quality embryos (G/AQE) in the present investigation. This investigation could conclude, similarly to others, that the presence of RC does not seem to affect an embryo's ability to create a GQE but does impair its ability to implant.

Absent cleavage has been characterized as a type of RC in a previous report (Liu *et al.*, 2014); however, in the current report it is classed as a distinct phenotype. The prevalence per embryo of this abnormality compared with RC is more than double (0.8% versus 0.4%) and of the four embryos that were transferred with this phenotype, one implanted. However, in a previous report of 22 embryos, none implanted that underwent type I or type II RC (defined here as AC) (Liu *et al.*, 2014). In another investigation using disaggregated human embryos, blastomeres were scored for the number of nuclei present after 16 to 20 h culture, and a small proportion of mononucleated blastomeres exhibited two nuclei after culture. It was postulated that approximately 30% of these occurred through AC (Pickering *et al.*, 1995). Here, AC was shown to occur in 1.08 embryos per treatment cycle and of the 133 embryos exhibiting AC, 122 were classed as PQE and 116 were discarded. Unlike DC, RC and CC, however, patient age was not shown to be significantly different when compared with the control embryo cohort.

CC has an overall prevalence per embryo of 5.3%: by far the highest of the five abnormal phenotypes, occurring in 1.82 embryos per treatment cycle, suggestive of a patient, treatment or environmental effect rather than a spontaneous event. One comprehensive analysis identified the prevalence of CC to be 15%, with a blastocyst formation rate of 14% and an IR of 0% (Athayde Wirka *et al.*, 2014). In the current analysis, one of the 48 transferred embryos implanted, a rate significantly lower than the IR of the control embryo cohort. Of the utilized embryos, just 18.2% were classed as GQE, 27.3% as AQE and 54.5% as PQE. Interestingly, it has previously been found that 35.2% of those exhibiting CC were classed as good quality, a result not synonymous with the current analysis. A possible explanation for this disagreement is the time-lapse technology used. In the current analysis, EmbryoScope® was the time-lapse technology of choice; however, in the analysis by Athayde Wirka *et al.* (2014) the Eeva™ system was used. The Eeva™ system uses dark field illumination to enable the software within it to track blastomeres, perhaps

making the distinction of blastomeres from fragments more difficult. An investigation conducted on patients carrying a Robertsonian translocation (the fusion of two acrocentric chromosomes), revealed that a high proportion of embryos resulting from these patients underwent numerous chaotic cleavage divisions, and rather than the aneuploid segregation of the Robertsonian translocation being the only reason for the infertility, there may be a post-zygotic manifestation leading to uncontrolled chromosome segregation (Conn *et al.*, 1998). The presence of chaotically dividing embryos has been noted elsewhere (Delhanty *et al.*, 1997; Harper and Delhanty, 1996; Laverge *et al.*, 1997) and has also been identified as a patient-related phenomenon (Delhanty *et al.*, 1997), evident in the current investigation in which CC occurred in up to 1.82 embryos per treatment cycle.

CL is largely discussed in the literature when considering frozen-thawed embryos and, as discussed previously, there is an associatively low IR (Tang *et al.*, 2006): 59.3% of the embryos were classed as PQE with 55.6% of the total discarded; just 13.6% were considered GQE and 27.2% AQE. As very few embryos were shown to exhibit this phenotype, and fewer still were transferred, it is difficult to draw conclusions about its implications. It would be reasonable to use previous evidence regarding frozen-thawed embryos to attribute their potential for success. However, CL in frozen embryos is likely to be a result of cryodamage during the freeze-thaw process, whereas in fresh embryos the CL could be as a result of exposure to another stressor such as suboptimal pH, temperature or osmolality. Cells that lyse may have a heightened sensitivity to changes in the environment, or lack a cytoplasmic constituent that regulates cell volume, for example, leading to lysis.

Abnormal phenotypes as deselection criteria

Where possible, UDC and TDC embryos should not be selected for transfer if other embryos are available, even when embryo quality is considered. It is important to note at this point that embryos transferred at the cleavage stage undergoing DC (of which there were five in the current analysis) will inevitably be classed as UDC. These embryos may have resulted in FDC; thus caution is advised owing to a potential bias in the current results of UDC cleavage-stage embryos. For this reason, extended culture of DC embryos may be valuable to allow the classification into either FDC or TDC and thus aid further in embryo selection and management of patient expectation. CC, the most common abnormal phenotype in the current analysis, has been linked to severe chromosomal abnormalities in the literature (Delhanty *et al.*, 1997), which could be patient specific. Therefore it is possible that the phenomenon could occur more than once in a patient cohort, indicating an underlying genetic condition. Where CC embryos are transferred, in the current analysis the IR is 2.1% regardless of embryo quality. For this reason, identification of CC as a deselection tool should be considered for laboratories utilizing time-lapse imaging technologies. Almost 92% of embryos that exhibited AC created PQE; thus they would be likely to be automatically discounted from clinical use. RC and CL each had an IR of 0%, albeit from low numbers of transferred embryos. However, the relative prevalence was low, the majority of

embryos exhibiting these phenomena were PQE and were not able to implant; therefore, these embryos should not be selected for transfer where possible. These recommendations have been implemented at the study site to aid in embryo selection. In addition to the above, the need for accurate and consistent annotation of embryos is imperative for any centre utilizing time-lapse technologies. This issue was raised a number of years ago, resulting in the publication of suggested terminology in order to create consensus among users (Ciray *et al.*, 2014). Consensus is paramount, and caution is advised when implementing or analysing time-lapse parameters discussed by others.

This preliminary investigation sought to determine the prevalence, implantation potential and suitability for inclusion in embryo selection algorithms of five abnormal cleavage events. To determine IR, only known implantation embryos were included, leading to a significant reduction in the number of embryos available for analysis. Nevertheless, this number would be difficult to achieve at another single site based on the study site using time-lapse imaging for all patients and performing over 2000 treatment cycles per year. In addition, the ability to track the implantation of these embryos is made more difficult with the increased likelihood of transferring two embryos in these cases, potentially due to reduced embryo quality in the available embryo cohort. Based on the results presented here, future analyses should focus on embryos undergoing more than one abnormal division event, the cell stage at which the abnormal cleavage event occurs, the effect of treatment parameters such as ICSI and day of transfer as well as the assessment of a relationship between the abnormal phenotypes and multinucleated blastomeres. In addition, the authors plan to perform an extension of this analysis to include embryo quality and outcome information regarding DC1–3 versus DC2–5. Finally, scrutiny should be paid to CL, with the specific timings of the CL event being assessed and linked to the relative impact on embryo viability.

In conclusion, embryos exhibiting an abnormal phenotype appear to have reduced developmental capability, expressed as both embryo quality and implantation potential. Time-lapse systems are bringing to light many unusual and, most likely, fundamentally complicated embryological phenomena, requiring in-depth analysis that could ultimately improve the outcome of treatment cycles.

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Table 1 Descriptive data for embryos showing an abnormal division pattern and normal embryos.

	No. of embryos n (%)	No. of cycles n (%)	Affected embryos/cycle	No. of embryos transferred	No. of embryos frozen	No. of embryos discarded	No. of GQE (%)	No. of AQE (%)	No. of PQE (%)	No. of abnormal embryos transferred with known implantation			IR % (n fhs)	P-value ^a
										Total	D3	D5		
TDC	48 (0.3)	45 (1.0)	1.07	3	11	34	10 (20.8)	8 (16.7)	30 (62.5)	1	0	1	0	NS
FDC	69 (0.4)	64 (1.4)	1.08	9	29	31	11 (15.9)	21 (30.4)	37 (53.6)	6	0	6	33.3 (2)	NS
UDC	580 (3.7)	463 (10.1)	1.25	33	70	477	69 (11.9)	101 (17.4)	410 (70.7)	16	5	11 (1xDET)	12.5 (2)	0.0378
Total DC	697 (4.4)	572 (12.5)	1.22	45	110	542	90 (12.9)	130 (18.7)	477 (68.4)	23	5	18 (1xDET)	17.4 (4)	NS
RC	65 (0.4)	61 (1.3)	1.07	14	22	29	15 (23.1)	10 (15.4)	40 (61.5)	9	3 (1xDET)	6	0	0.0153
AC	133 (0.8)	123 (2.7)	1.08	7	10	116	6 (4.5)	5 (3.8)	122 (91.7)	4	1	3 (1xDET)	25 (1)	NS
CC*	835 (5.3)	459 (10.1)	1.82	85	69	681	28 (18.2)	42 (27.3)	84 (54.5)	48	25 (5xDET)	23 (6xDET)	2.1 (1)	<0.0001
CL	81 (0.5)	71 (1.6)	1.14	5	31	45	11 (13.6)	22 (27.2)	48 (59.3)	2	0	2	0	NS
Overall	1811	1286	1.41	156	242	1413	-	-	-	86	34	52	7.0	<0.0001

total	(11.4)	(28.2)									(6xDET)	(8xDET)	(6)	1
Normal embryos	14008 (88.6)	3273 (71.8)	-	3456	4574	5978	5128 (36.6)	2468 (17.6)	6412 (45.8)	-	-	-	38.7 (1336)	-

Total DC constitutes TDC, FDC and UDC collectively.

AQE = average quality embryos; GQE = good quality embryos; PQE = poor quality embryos.

^a For implantation rate (IR) of affected embryos compared with normal embryo cohort (Fisher's exact, significant at $P < 0.05$).

* Only transferred and frozen embryos assessed for quality for this category owing to significant missing data.

Table 2 Baseline characteristics for cycles with embryos undergoing an abnormal division pattern and cycles with normal embryos.

	Patient age (mean \pm SD)	<i>P</i> -value	Oocytes collected (mean \pm SD)	<i>P</i> -value	Previous attempts (mean \pm SD)	<i>P</i> -value
TDC	32.82 \pm 4.7	<0.0001	12.95 \pm 7.78	<0.0001	1.37 \pm 0.93	NS
FDC						
UDC						
RC	32.5 \pm 4.5	0.0097	15.7 \pm 9.7	<0.0001	1.23 \pm 0.6	NS
AC	33.16 \pm 5.41	NS	15.09 \pm 8.57	<0.0001	1.35 \pm 0.8	NS
CC	32.93 \pm 4.87	<0.0001	13.44 \pm 8.5	<0.0001	1.39 \pm 0.82	NS
CL	33.24 \pm 4.27	NS	13.86 \pm 7.79	<0.0001	1.28 \pm 0.78	NS
Total abnormal	32.93 \pm 4.75	<0.0001	14.21 \pm 8.47	<0.0001	1.32 \pm 0.79	NS
Normal	34.08 \pm 4.73	-	10.5 \pm 5.99	-	1.37 \pm 0.98	-

P-values for cycles with abnormal embryos versus cycles with normal embryos (Student's *t*-test, significant at *P* < 0.05).

AC = absent cleavage; CC = chaotic cleavage; CL = cell lysis; FDC = false direct cleavage; NS = not statistically significant; RC = reverse cleavage; TDC = true direct cleavage; UDC = unconfirmed direct cleavage.