Title

A preliminary investigation into the prevalence and implantation potential of five abnormal embryonic phenotypes assessed using time lapse imaging.

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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 direct cleavage (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 create a pregnancy has been shown to be significantly reduced (Rubio et al., 2012) where 13.7% (715/5225) of

all examined embryos and 6.6% (109/1659) of transferred embryos underwent DC, with 1.2% (1/109) resulting in a clinical pregnancy. These embryos have been shown to have a markedly decreased blastocyst formation rate when compared to their normal counterparts (Athayde Wirka et al., 2014).
The second abnormal phenotype to be considered is reverse cleavage (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 to their non reverse cleaved counterparts (Hickman et al., 2012). This research concluded that RC does not seem to impair embryo development to the blastocyst stage supported by the findings of others (Desai et al., 2014).
Absent cleavage (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 categorised under RC, termed type II RC (Liu et al., 2014). Of those embryos that underwent RC (27.4%, (216/789), 82% were classed as type II; absent cleavage rather than blastomere fusion. Further evidence of this specific developmental pattern has not yet been published perhaps due to the likelihood that these embryos will be used for treatment.
Chaotic cleavage (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 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 cell lysis (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 embryo. However, if CL accounted for 25 to 50% of the embryo the pregnancy rate was 3.2%; significantly lower than if less than 25% CL had occurred (16.6%) (Tang et al., 2006) 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 with regards to the prevalence and implication of the presence of certain abnormal phenotypes. Further investigation into these phenomena is required to determine if their presence is severe enough 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) as well as Institutional Review Board approval. All procedures and protocols complied with UK regulation (Human Fertilisation and Embryology Act, 1990, 2008). Data were obtained from 5131 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 agonist (buserelin, Suprecur®, Sanofi Aventis, UK) or antagonist (cetrorelix acetate, Cetrotide®, Merck Serono, Germany). Ovarian stimulation was performed using urine derived or recombinant follicle stimulating hormone (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 5000IU of subcutaneous hCG (Gonasi® HP, IBSA Pharmaceuticals, Italy) 36 hours prior to oocyte collection. Luteal support was provided using 400mg 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 4 well dishes (Nunc™, Thermo Scientific, USA) each well containing 0.65ml GIVF™ (Vitrolife, Gothenburg, Sweden) covered with 0.35ml 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 ten minutes followed by two washes at 0.6rcf for ten minutes using GIVF™. Those oocytes destined for ICSI were prepared using enzymatic (HYASE 10X™, Vitrolife) and mechanical digestion. ICSI was performed on all metaphase II oocytes (MII) approximately four hours following collection after which time all injected oocytes were placed in individual culture drops of G1™ (for all cycles pre September 2014) or GTL™ (all cycles post September 2014) (Vitrolife) and cultured in the EmbryoScope® (Vitrolife). Those oocytes destined for standard insemination (IVF) had this performed approximately four hours after collection and were replaced into a standard incubator until fertilisation check the following day. Oocytes were then checked for fertilisation approximately 16 to 18 hours post insemination (hpi) and all fertilised oocytes along with all unfertilised metaphase II 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)) along with an internally derived, ESA. This ESA 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-. Embryo transfer was performed using the highest

grade embryo(s) either three or five days post collection depending on the number of good quality embryos the patient had on day three as well as how many were to be transferred. Selected embryos were cultured in EmbryoGlue® (Vitrolife) for 10 to 30 minutes in a standard incubator prior to embryo transfer. Embryos were cultured at 37°C, 6% CO_2, 5% O_2, 89% N_2 throughout.

Analysis of time lapse information
The image interval on the EmbryoScope® was set to 10 minutes with seven focal planes. Images were collected for the duration of culture immediately following ICSI or fertilisation check (for IVF derived embryos) to utilisation. 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 categorisation or the cessation of culture before the morula stage was reached (supplementary figure 1).

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 their quality on the day of utilisation defined as good, average or poor (supplementary table 1). The IR for each abnormal phenotype was determined where the origin of the fetal heart could be confirmed i.e. using known implantation data from an abnormal embryo or not. 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 the student 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 statistically 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 5131 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. These embryos resulted in 3456 embryos transferred and 1336 fetal heartbeats (IR= 38.66%) (table 1). 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 4.38% (TDC, FDC, UDC, collectively) and 5.25%, respectively. The remaining phenotypes had considerably lower prevalence ranging from 0.41 to 0.84% (table 3). The overall prevalence per embryo observed of abnormal division patterns was 11.39% (table

3). The IR of abnormal embryos ranged from 0 to 33.3% (table 3). Of the five abnormal division patterns the IR of UDC, CC and RC were significantly lower than normal counterparts; 12.5% (2/16), 2.1% (1/48) and 0% (0/9), respectively (table 3). Furthermore, the overall IR of all abnormal embryos was statistically significantly lower than normal counterparts (6.9% (6/86) vs 38.66%) (table 1 and 3). In all cases the percent of good quality embryos resulting from those exhibiting abnormal division patterns never reached above 24% and the majority of embryos were classified as poor quality (table 3). This is also reflected in the utilisation of these embryos where the highest proportion of each group was discarded (supplementary figure 2).

Patient age was statistically significantly lower for those undergoing DC, RC and CC to those not exhibiting an abnormal division pattern. The number of oocytes collected was found to be statistically significantly higher in treatment cycles containing abnormal embryos than those not containing embryos exhibiting an abnormal division pattern. Finally, the number of previous attempts was not found to be statistically significantly different between any of the abnormal division categories and the control embryo cohort (table 2). Baseline information from treatment cycles containing an abnormal embryo did not contribute to baseline information for the control cohort.

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 4.38% (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 statistically significantly lower than that of the control embryo cohort although this could be attributed to the reduced numbers. A classification system of DC was not adopted by other publications therefore if FDC were not considered, the IR would be statistically 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. Genetic assessment of DC oocytes has revealed three division patterns; DC to three cells (62%); cleavage to a morphologically normal two cell ‘embryo’ (24%) and cleavage to a two cell ‘embryo’ plus an extrusion (14%) (Kola et al., 1987). All triploid 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 two 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 two cell ‘embryo’ plus an extrusion, 75% were found to have two diploid blastomeres and a haploid extrusion. In the analysis presented here, the IR of FDC, those embryos analogous to the two cell embryo plus an extrusion, was 33.3% (2/6). Caution should be taken as the numbers are very reduced 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. The findings by Kola et al. (1987) may indicate that embryos could 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 trisomy embryos correct more often than other aneuploidies (Barbash-Hazan

et al., 2008) possibly occurring through the loss of a chromosome in trisomy 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 to previous reports may also be due to observers having experience with the different categorisations of DC, making them proficient at recognising patterns of FDC, such as blastomere behavior, 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 where 26 were classed as good or average quality. It is likely that embryos classed as PQE were utilised due to unavailability of others. The IR of embryos undergoing RC in the current investigation was 0% (0/9). 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 recognised 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. This analysis included 1141 embryos frozen on day two and 873 frozen on day three. RC was found in 51 embryos of which 70% were classed as good quality. The overall frequency of RC was 4.6% in day two embryos and 1.5% in day three embryos. A slightly higher incidence of blastomere fusion was found in embryos created using IVF when compared to ICSI. When a control group was observed (embryos not subject to freezing and thawing) the prevalence of RC was 0.3%, a result not far from that recorded in the present study (0.41%). 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 a single live birth only. Again, a result similar to that seen in the present investigation where no pregnancies resulted from nine embryos transferred that had undergone RC. The chromosomal status of blastomeres resulting from fusion was also examined where embryos affected by RC were transformed into either polyploidy or mosaics 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% G/AQE in the present investigation. This investigation could conclude similarly to others where the presence of RC did not seem to affect an embryos ability to create a GQE but does impair an embryos ability to implant.

Absent cleavage has been characterised 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 to RC is more than double (0.84 vs 0.41%) 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 20h culture and a small proportion of mononucleated blastomeres exhibited two nuclei after culture. It was hypothesised 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, the patient age was not shown to be significantly different when compared to the control embryo cohort.

CC has an overall prevalence per embryo of 5.25%; 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, the IR of these embryos was 2.1% (1/48); statistically significantly lower than the IR of the control embryo cohort. Of the transferred embryos, just 4.7% were classed as GQE, 22.4% as AQE and 72.9% 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. The EmbryoScope® does not use dark field illumination which could make distinction of blastomeres from fragments more straightforward. 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) a statement synonymous with CC occurring 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). 59.2% of the embryos were classed as PQE with 55.6% of the total discarded. Just 13.6% were considered GQE and 27.2% AQE, a result similar to other abnormal phenotypes. As very few embryos were shown to exhibit this phenotype, and fewer still were transferred, it is difficult to draw conclusions about the implications of this abnormal phenotype. 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 as 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 its 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. CC, the most common abnormal phenotype in the current analysis, has been linked to severe chromosomal abnormalities in the literature which could be patient specific therefore it's possible that the phenomenon could occur more than once in a patient cohort indicating an underlying genetic condition. Where CC embryos are transferred the expected IR is 2.1% regardless of embryo quality. For this reason, identification of CC as a deselection tool should be considered for laboratories utilising time lapse imaging technologies. Just fewer than 92% of embryos that exhibit AC create PQE thus they would likely be automatically discounted from clinical use. RC and CL each have an IR of 0%, albeit from low numbers of transferred embryos. However, the relative prevalence is low, the majority of embryos exhibiting these phenomenons are PQE and they are 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.

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 used 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 for all patients and performing over 2000 treatment cycles per year. 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 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.

Appendix: Supplementary material

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Total embryos (n)	14008
Embryos transferred (n)	3456
Embryo transfers (n)	3273
Sum fhs (n)	1336
Count fhs (n)	1269
IR (%)	38.66
CPR (%)	38.77

Table 1. Baseline information for embryos not exhibiting an abnormal division pattern Including total number of embryos, number of embryos transferred, number of embryo transfers, total fetal heartbeats (fhs), count of fhs (regardless of number), implantation rate (IR), clinical pregnancy rate (CPR). IR was calculated as sum fhs/embryos transferred. CPR was calculated as count fhs/embryo transfers.

	Affected embryos (n)	Treatment cycles (n)	Patient age (mean ± S.D)	p-value	Oocytes collected (mean ± S.D)	p-value	Previous attempts (mean ± S.D)	p-value
TDC	48	45	32.82 ± 4.7	<0.0001	12.95 ± 7.78	<0.0001	1.37 ± 0.93	>0.05
FDC	69	64						
UDC	580	463						
RC	65	61	32.5 ± 4.5	0.0097	15.7 ± 9.7	<0.0001	1.23 ± 0.6	0.2663
AC	133	95	33.16 ± 5.41	0.0629	15.09 ± 8.57	<0.0001	1.35 ± 0.8	0.8438
CC	835	459	32.93 ± 4.87	<0.0001	13.44 ± 8.5	<0.0001	1.39 ± 0.82	0.6765
CL	81	71	33.24 ± 4.27	0.1381	13.86 ± 7.79	<0.0001	1.28 ± 0.78	0.4422
Normal	14008	3273	34.08 ± 4.73	-	10.5 ± 5.99	-	1.37 ± 0.98	-

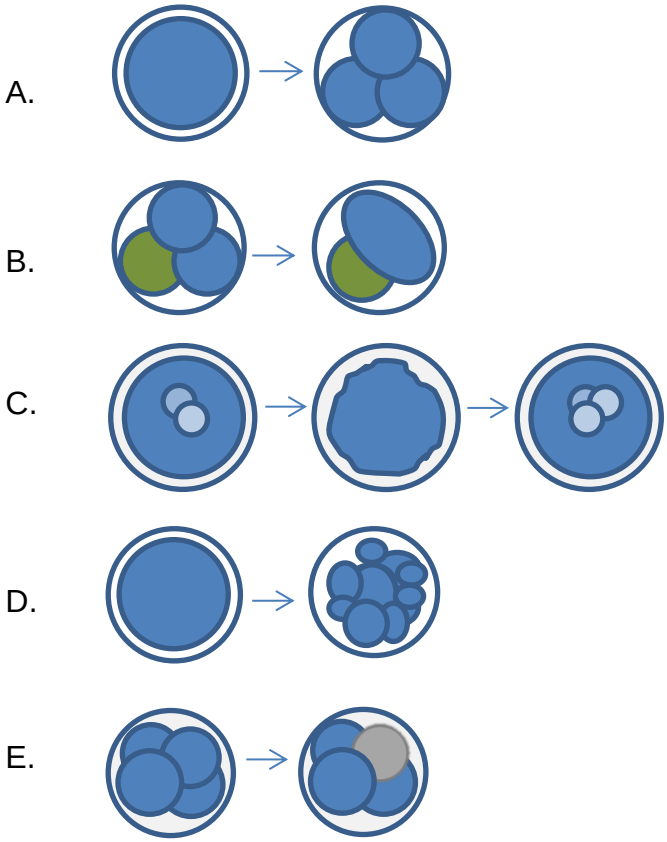
Table 2. Baseline information for embryos undergoing an abnormal division pattern including the total number of affected embryos, the number of treatment cycles these pertain to, the mean patient age, oocytes collected and previous attempts. The mean patient age, oocytes collected and previous attempts were statistically analysed against the normal embryo cohort for significant differences (student t-test, significant at $p<0.05$).

	Embryos (n)	Cycles (n)	Affected embryos/cycle	Prevalence/ embryo (%)	Prevalence/cycle (%)	Transfer	Freeze	Discard	GQE (n (%))	AQE (n (%))	PQE (n (%))	Abnormal embryos transferred (n)	FHS (n)	IR (%)	p-value
TDC	48	45	1.07	0.3	0.9	3	11	34	10 (20.8)	8 (16.7)	30 (62.5)	1	0	0	>0.05
FDC	69	64	1.08	0.43	1.2	9	29	31	11 (16.0)	21 (30.4)	37 (53.6)	6	2	33.3	>0.05
UDC	580	463	1.25	3.65	9.0	33	70	477	69 (11.9)	101 (17.4)	410 (70.7)	16	2	12.5	0.0378
DC	697	572	1.22	4.38	11.1	45	110	542	90 (12.9)	130 (18.7)	477 (68.4)	23	4	17.4	0.05
RC	65	61	1.07	0.41	1.2	14	22	29	15 (23.1)	10 (15.4)	40 (61.5)	9	0	0	0.0153
AC	133	123	1.08	0.84	2.4	7	10	116	6 (4.5)	5 (3.8)	122 (91.7)	4	1	25	>0.05
CC*	835	459	1.82	5.25	8.9	85	69	681	4 (4.7)	19 (22.4)	62 (72.9)	48	1	2.1	<0.0001
CL	81	71	1.14	0.51	1.4	5	31	45	11 (13.6)	22 (27.2)	48 (59.2)	2	0	0	0.5257
Overall	1811	1286	1.41	11.39	25.0	156	242	1413	-	-	-	86	6	6.9	<0.0001

Table 3. Descriptive data regarding embryos that underwent an abnormal division pattern. The total number of affected embryos, number of affected treatment cycles, the number of affected embryos per treatment cycle, prevalence per embryo (defined as number of affected embryos/total number of embryos), prevalence per cycle (defined as number of affected treatment cycles/total number of treatment cycles), their fate, their quality and the IR of transferred embryos that were abnormal is shown. The IR of these embryos was then compared to that of the normal embryo cohort for statistical significance (Fisher's exact, significant at $p<0.05$).

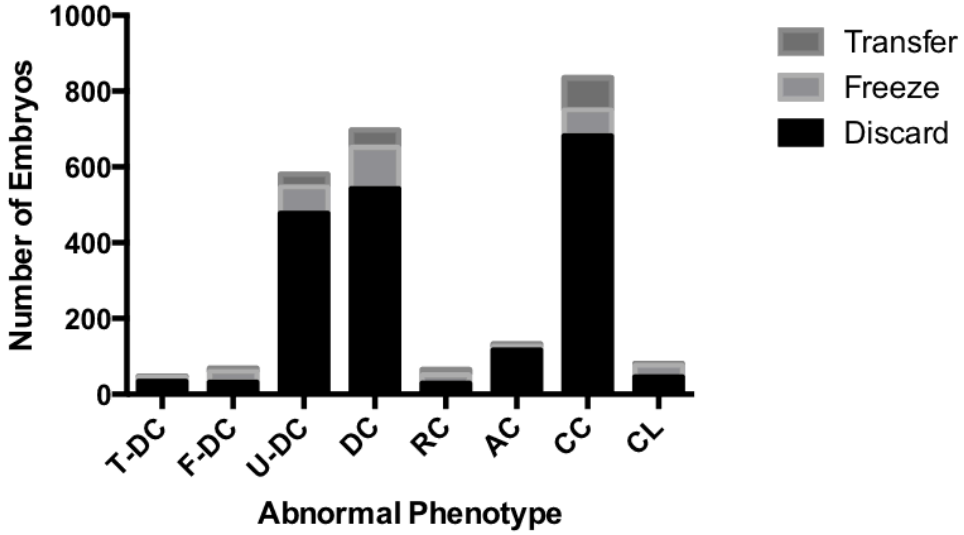
*only transferred embryos assessed for quality for this category due to significant missing data.

Supplementary figure 1



Supplementary figure 1; a schematic representation of five categories of abnormal embryo phenotypes. A. Direct cleavage; cleavage of one blastomere into three distinct blastomeres. B. Reverse cleavage; the fusion of two blastomeres into a single blastomere. C. Absent cleavage; pronuclear/ nuclear fading followed by a cytoplasmic ‘roll’, no division, but an additional, or multiple, nuclei. D. Chaotic cleavage; cleavage of one cell into multiple fragments with no discernable blastomeres. E. Cell lysis; the lysing of one blastomere within an embryo at any stage of development.

Supplementary figure 2



Supplementary figure 2. Fate of embryos exhibiting abnormal phenotypes. Proportion of embryos transferred, frozen or discarded that underwent an abnormal division pattern where direct cleavage (DC) includes true direct cleavage (TDC), false direct cleavage (FDC) and unconfirmed direct cleavage (UDC) combined. RC; reverse cleavage, AC; absent cleavage, CC; chaotic cleavage, CL; cell lysis.

Supplementary table 1

Good quality embryos (GQE)	Cleavage stage embryos with even blastomeres (<20% difference in diameter) and <20% fragmentation Blastocyst embryos with prominent and compact inner cell mass and many cells forming a cohesive epithelium
Average quality embryos (AQE)	Cleavage stage embryos with 20-50% difference in cell diameter and/or 20-50% fragmentation Blastocyst stage embryos with easily discernable inner cell mass with many cells that are loosely grouped together
Poor quality embryos (PQE)	Cleavage stage embryo with >50% difference in blastomere diameter and/or >50% fragmentation Blastocyst stage embryos with few cells forming the inner cell mass and very few cells making up the trophectoderm
Definitions of embryo quality used to classify embryos as good, average and poor quality based on ACE/BFS embryo grading guidelines (Cutting et al., 2008).	

Supplementary table 2

	Abnormal embryos transferred (n)	Total transfers (n)	SET (n)	DET (n)	Cleavage stage transfers (n)	Blastocyst stage transfers (n)
TDC	1	1	1	0	0	1
FDC	6	6	6	0	0	6
UDC	16	15	14	1	5	10
DC	23	22	21	1	5	17 (1xDET)
RC	9	8	7	1	2 (1xDET)	6
AC	4	3	2	1	1	2 (1xDET)
CC	48	37	26	11	20 (5xDET)	17 (6xDET)
CL	2	2	2	0	0	2
Overall	86	72	58	14	28	44

Embryo transfer baseline information for each abnormal embryo phenotype including the total number of transfers, the number of single embryo transfers (SET), double embryo transfers (DET), cleavage stage transfers and blastocyst stage transfers.