Contraction behaviour reduces embryo competence in high-quality euploid blastocysts

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Abstract

Purpose The aim of the study is to investigate how blastocyst contraction behaviour affects the reproductive competence in high-quality euploid embryos.

Methods Eight hundred ninety-six high-quality blastocysts derived from 190 patients (mean age 38.05 (SD = 2.9) years) who underwent preimplantation genetic testing for aneuploidies (PGT-A) from January 2016 to October 2017 were included in this study. PGT-A results were reported as euploid or aneuploid. Aneuploid embryos were sub-classified into three categories: monosomy, trisomy and complex aneuploid. Retrospective studies of time-lapse monitoring (TLM) of those embryos were analysed and reproductive outcome of transferred embryos was collected.

Results A total of 234/896 were euploid (26.1%) whilst 662/896 (73.9%) blastocysts were proven to be aneuploid from which 116 (17.6%) presented monosomies, 136 (20.5%) trisomies and 410 (61.9%) were complex aneuploid. The most frequent chromosomal complements were trisomies affecting chromosome 21 and monosomies involving chromosomes 16 and 22. Data analysis showed a statistical difference in the number of contractions being reported greater in aneuploid when compared to euploid embryos (0.6 vs 1.57; $p < 0.001$). Analysis of the aneuploid embryos showed that monosomies present less number of contractions when compared to embryos affected with trisomies or complex aneuploidies (1.23 vs 1.53 and 1.40; $p < 0.05$). No difference was observed when comparing the latter two groups. Euploid embryos presenting at least one contraction resulted in lower implantation and clinical pregnancy rates when compared to blastocysts that do not display this event (47.6 vs 78.5% and 40.0 vs 59.0% respectively).

Conclusions Most aneuploid blastocysts diagnosed by PGT-A have complex aneuploidies, showing that aneuploid embryos can develop after genomic activation and reaching high morphological scores. It becomes clear that embryo contraction, despite being a physiological feature during blastulation, is conditioned by the ploidy status of the embryo. Furthermore, the presence of contractions may compromise implantation rates.

Keywords Blastocyst contraction · Next-generation sequencing · Time-lapse monitoring · Preimplantation genetic testing for aneuploidies · Ploidy

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Introduction

Time-lapse monitoring (TLM) incubation systems have allowed embryologists to study the dynamic process of embryo development, from fertilisation to the formation of the blastocyst. TLM technology marked a real turning point since it allows embryos to be scored not only by their morphological features but also by their development kinetics [1–3]. Along with the concept of continual embryo monitoring, advances in genetic testing in recent years have helped to refine embryo selection and increase live birth rates per embryo transfer [4]. Recently, next-generation sequencing (NGS) has become an

important tool for clinical diagnosis after comparative genomic hybridisation (aCGH), the current gold standard, increasing the detection rate and analysis resolution of all human chromosomes [5–7]. Reproductive success is thought to be related to embryo ploidy status. An increase in embryo aneuploidy rate decreases the chances of a successful pregnancy outcome [8, 9]. Interestingly, the aneuploidy rate has been reported in patients under 35 to be around 44.9%, in patients who were recommended elective single embryo transfer (eSET) based only on embryo morphology [7].

There have been several attempts to create an algorithm, with the aid of TLM, which would help embryologists select the embryo with the highest implantation rate. Correlation with ploidy status of the embryo suggested that such parameters could not be used to select euploid blastocysts in poor-prognosis patients [10–12]. To date, data supporting the use of TLM is limited, and it is unclear whether selecting embryos using TLM is better than conventional morphology criteria. For further understanding of embryo development, new parameters are being studied. Lewis and Gregory [13] first described contraction during the blastocyst stage observed in various mammals as an event in embryo development where the perivitelline space is observed by the retraction of trophoctoderm (TE) cells [28]. The physiological role and clinical significance of this observation remains to be fully understood. Niimura [14] described this phenomenon as being related to the ability of embryos to hatch out from the zona pellucida [29]. A few studies have included the contraction pattern in an algorithm for embryo selection. A retrospective study with recipients of oocyte donation and autologous IVF cycles showed some kinetic parameters such as time to reach morula stage and blastulation to be significantly shorter in those embryos that have contractions in their development [15]. Results indicated a reduction of implantation rate from 48.5 to 35.1% in those blastocysts that displayed at least one contraction. Another study analysed 38 embryos from egg donation cycles with known implantation outcome [16]. In that study, abnormal kinetics of blastocyst expansion and slower blastulation were described in embryos that present contractions. This in turn had negative impact on implantation. In a more recent study, lower live birth rates were reported after transferring frozen blastocysts when the number of contraction events was increased. However, multivariate analysis revealed female age as a confounder [17].

This study aims to understand blastocyst contraction patterns with the aid of TLM technology correlating these patterns and different genetic complements exhibited by human embryos. To our knowledge, this is the first attempt to investigate this event in embryos with different genetic contents

(i.e. euploidy, monosomies, trisomies and complex aneuploidies) and correlate that with reproductive outcome.

Material and methods

This retrospective observational study was conducted at the Centre for Reproductive and Genetic Health in London, UK. A database was generated in order to retrospectively analyse embryos that were cultured in the Embryoscope time-lapse system for preimplantation genetic testing for aneuploidy (PGT-A). All patients who underwent conventional ovarian stimulation during January 2016 to February 2017 and had PGT-A carried out on their embryos were considered. Natural IVF cycles, cycles where the embryos were thawed on day 3 for blastocyst culture or frozen oocyte cycles were excluded. The retrospective observational study design did not require ethical approval for the use of human subjects.

Study cohort

A cohort study of 190 patients was analysed. The mean age of the patients was 38.05 ± 2.9 years. A total of 2350 oocytes were retrieved and 1596 2PN embryos were obtained. After 5–6 days of culture, 1368 blastocysts were considered suitable for biopsy (Supplemental Fig. 1). In order to eliminate any bias generated from quality-dependent variables during the clinical outcome analysis, only embryos graded as high-quality were included (896 blastocysts).

Embryo quality and grading system

Embryos were generated through intracytoplasmic sperm injection (ICSI) or intracytoplasmic morphologically selected sperm injection (IMSI) and cultured until the blastocyst stage (day 5–6), as standard procedures for the program. Embryos were graded according to modified Gardner and Cornell's group scoring system [18, 19]. For the inner cell mass (ICM), grade depends on cellularity and cell clustering and can be distinguished in the following: A, tightly packed and compacted cells; B⁺, larger cells, not tightly packed or cells making up a cellular bridge; B[−], ICM visible but loose and/or fragmented cells; C, no ICM distinguishable; and D, when cells of ICM appear degenerative. Similarly, different grades can describe the TE cells: A, many healthy cells forming a cohesive epithelium; B⁺, moderate levels of cells, but healthy and larger in size; B[−], few large but healthy cells; C, poor, very large or unevenly distributed cells and may appear as few cells squeezed to the side; and D, when TE cells appear degenerative. For embryos to be considered “high-quality”, the following requirements were needed: tightly packed and compacted cells defining the ICM (graded as A or B⁺) and a highly

cellular structure forming a cohesive structure described for the TE (A or B⁺).

Blastocyst biopsy and PGT-A

Blastocyst biopsy was performed on days 5 and 6 of development using standard biopsy methods [20] and blastocysts were cryopreserved using Cook Blastocyst vitrification media as described by Stojanov et al. (2009). All blastocysts obtained were biopsied only once and a minimum of five cells were taken. Two different outcomes were considered after the PGT-A testing: euploid and aneuploid. Mosaic embryos (*described in the section below*) were not included in the dataset since the proportion found in high-quality embryos was less than 2% (16/912) (Supplemental Fig. 1). As per the unit policy, mosaic embryos were not transferred; therefore, they were excluded from the analysis. Advances in genetic testing and resolution of the techniques are continuously improving. Therefore, it is important to consider that some of the embryos included in the study are still likely to be mosaic, but not detected.

To understand the individual chromosome alterations, the sample was divided into three aneuploid groups: (a) monosomies, where only complete loss of one or more chromosomes was described; (b) trisomies, consisting of embryos displaying complete gains of one or more chromosomes; and (c) complex aneuploid when the genetic profile included chromosomes affected by a combination of gains, losses and partial alterations. Embryos with subchromosomal abnormalities presented further alterations and were included in the latter group (and so were embryos whose all chromosomes were affected simultaneously). The method of PGT-A testing was NGS. Prior to blastocyst biopsy, laser-assisted zona hatching (AH) was performed in all embryos on day 3 in order to facilitate the biopsy at blastocyst stage.

Next-generation sequencing

Genetic testing was carried out externally with the help of the corresponding genetic testing provider. NGS technology for aneuploidy detection uses 36-bp single read end sequencing on an Illumina MiSeq. Sample processing followed the manufacturer's instructions: VeriSeq PGS protocols from Illumina. The resolution of the assay could be as low as 5 MB. However, it also strongly depends on the genomic region and therefore can slightly vary per genomic location. After next-generation sequencing, BAM files were generated and imported into the Bluefuse Multi software for analysis. BAM files contain generic alignment format for storing read alignments against reference sequences in this case human genome. Filtering algorithms only use reads that align uniquely to the human genome, are not duplicates of other reads and reach a sufficient base quality. Algorithms calculate and call the status for each chromosome as either euploid or aneuploid

and include an estimate of confidence in the call based on assay noise or underlying ambiguity. Typically, <0.1% of the genome is sequenced. Mosaicism could be detected if one out of five cells (20%) has a different karyotype to the others. An embryo was classified as mosaic when 30–80% of the cells had a different chromosomal complement.

Time-lapse monitoring incubator

Mature oocytes retrieved were inseminated by ICSI or IMSI and subsequently placed in pre-equilibrated slides (EmbryoSlide, Vitrolife). Embryoscope was used to perform all the annotations and measurements accordingly with the most updated criteria [21]. Five plane focal images were taken and recorded every 15 min for 140 h. All annotations were performed retrospectively using embryo viewer software by two embryologists. All embryologists involved have been validated on their annotation accuracy by taking part in internal laboratory quality assurance exercises on a quarterly basis as well as external quarterly quality control on a national level. Sage Single Step Media (Origio, UK) was used and not replaced during the embryo culture.

Blastocyst contraction

Blastocyst contraction was defined according to the methodology described by Marcos et al. (2015) as an event in embryo development during blastulation, when the perivitelline space is observed again by the retraction of TE cells (Fig. 1). Contraction pattern was studied from the time when the TE cells started pushing on the zona pellucida (ZP), as described previously by Bodri et al. (2016). The presence and number of embryo contractions was annotated for each embryo individually and analysed blindly to the corresponding genetic result.

Embryo thawing and transfer

Euploid blastocysts were thawed using Cook Blastocyst Thawing media as described by Stojanov [22] and transferred individually in a frozen medicated cycle [30]. For the period studied, one single embryo transfer per patient with suitable embryos was registered. Only euploid embryos were considered for transfer.

Outcome measures and statistical analysis

Statistical calculations were performed with Statistical Package for the Social Sciences version 23 (SPSS Inc., Chicago, IL). The main outcome was to observe any correlation between contraction behaviour (contraction presence) and clinical outcomes after euploid embryo replacement in single embryo transfer cycles. Analysis of variance (ANOVA) was required for continuous data when comparing both groups.

Fig. 1 Embryo contraction presence during blastulation. Contraction behaviour is annotated during blastulation, from the moment when the trophoblast cells start pushing towards the zona (Fig. 1 100.6 h).

Contraction presence was described by the retraction of TE cells (Fig. 1 102.3 h). Embryo biopsy is performed on fully expanded blastocysts with hatching cells (Fig. 1 117.3 h)

Normal distribution was tested using Shapiro-Wilk test and continuous variables were compared using the Student's *t* test. The chi-square test was used to analyse nominal/categorical variables and percentages. Pregnancy rate was calculated considering the total positives (b-HCG) out of the total number of embryo transfers. Clinical pregnancy rate was defined as the presence of a positive heartbeat out of the total number of embryo transfer cycles under the same category. To confirm the findings, clinical pregnancy rate was then fitted to a logistic regression considering the subsequent covariates: presence of contractions (yes or no), female age (years), body mass index (kg/m^2), number of mature oocytes (MII) and utilised blastocysts. Significance for statistical analysis was fixed at a 5% level.

Results

Summarised in Table 1 are the baseline cycle characteristics in groups where contraction is observed or not. The total number of high-quality blastocysts analysed was 896, of which 504/896 (56.2%) presented contractions during their development, including 227/504 (45.1%) with multiple contraction patterns. A total of 392/896 (43.7%) of the analysed blastocysts did not display the event. Among the included embryos, 662/896 (73.9%) blastocysts proved to be chromosomally abnormal, whilst 234/896 (26.1%) were chromosomally normal.

Genetic complements in aneuploid embryos

Embryos presenting with complex aneuploidies were the most frequent in the aneuploid group (61.9%; 410/662), followed by trisomies (20.5%; 136/662) and monosomies (17.6%; 116/662). In Fig. 2, the different chromosomal complements of aneuploid embryos across the sample are described. For each chromosome, different anomalies had been taken into account: gain or loss of genetic material (leading to a trisomy

or monosomy for that chromosome, respectively) and partial gain or loss of chromosomes (when only part of the chromosome is involved in the alteration). The frequency of complete gain was most frequently seen in chromosome 22, followed by chromosomes 21 and 14 respectively. In contrast, chromosomes 15 and 16 were identified as the most common chromosomes to lose one copy. Furthermore, chromosome 1 was found to be sensitive to partial gains and losses across the sample. As illustrated in Supplemental Table 1, analysis of individualised aneuploid categories for each chromosome was performed. Looking at the chromosomes leading to complex aneuploidies, chromosome number 22 was identified in more than 20% of the cases.

Embryo biopsy day and euploidy

Embryo biopsy was predominantly performed on day 5 (65.5%; 587/896) compared to day 6 (34.5%; 309/896). Both groups revealed a similar contraction pattern (Table 1). Analysis of euploidy rate showed no statistical difference between day 5 or 6 high-quality embryos in both groups separately (no contractions, 35.7% D5 vs 32.2% D6 *p* value 0.098; contraction presence, 21.4% D5 vs 16.2% D6 *p* value 0.107). Chi-square analysis further revealed that the euploidy rate was reported significantly lower in embryos that showed contractions regardless of the biopsy day (Table 1).

Embryo contraction behaviour and aneuploidy

A chi-square test of independence was used to compare the presence of contraction in both groups. Analysis of the data showed that this event was predominantly present in aneuploid embryos ($p < 0.001$). A total of 98/234 (41.8%) among the euploid ones present contractions during their development whereas this feature was seen in 406/662 (61.3%) of the aneuploid group. Moreover, embryos with abnormal genetic complement revealed a significantly higher number of

Table 1 Baseline characteristics of the cohort studied. There was no statistical significant difference among baseline variables (female/male age, BMI, basal AMH/FSH) or laboratory parameters such as oocyte maturity, fertilisation or blastocyst utilisation. *MII*, metaphase II; *2PN*, two pronuclei

	No contractions (<i>n</i> = 392)	Presence of contractions (<i>n</i> = 504)	<i>p</i> value
Female age (years)	38.1 ± 1.8	38.8 ± 2.4	0.136 ^a
Male age (years)	40.3 ± 6.6	41.6 ± 8.1	0.370 ^a
Body mass index (kg/m ²)	24.4 ± 4.4	23.1 ± 3.8	0.358 ^a
Basal FSH (mIU/ml)	6.1 ± 1.4	6.6 ± 2.2	0.381 ^a
Basal AMH (ng/ml)	25.8 ± 16.2	22.3 ± 18.1	0.091 ^a
Oocytes retrieved (<i>n</i>)	14.5 ± 6.5	14.3 ± 7.8	0.786 ^a
Mature oocytes	12.1 ± 5.1	11.8 ± 5.9	0.612 ^a
Oocytes fertilised (<i>n</i>)	9.5 ± 4.8	9.7 ± 4.2	0.811 ^a
Utilised blastocysts (<i>n</i>)	5.9 ± 3.1	6.1 ± 2.6	0.751 ^a
Biopsy day (% , <i>n</i>)			0.917 ^b
Day 5	69.9% (274)	62.2% (313)	
Day 6	30.1% (118)	37.8% (191)	
Euploidy (% , <i>n</i>)			0.681 ^b
Day 5	35.7% (98)	21.4% (67)	
Day 6	32.2% (38)	16.2% (31)	

^a Student's *t* test

^b Chi-square test

contractions when compared to euploid embryos (0.60 vs 1.57, $p < 0.001$). Contraction behaviour was further assessed in aneuploid embryos only considering the genetic complement (monosomies, trisomies or complex aneuploidies). The student's *t* test indicated a statistical difference in the number of contractions of embryos presenting monosomies when compared to embryos exhibiting trisomies or complex aneuploidies (1.23 vs 1.53 and 1.40; $p < 0.05$) illustrated in Fig. 3. No difference was reported between aneuploid trisomies and complex aneuploidies with regard to the number of contractions ($p = 0.348$). Descriptive analysis of the data showed that some

chromosomes displayed different alterations when comparing the genetic complements between embryos that present contractions during their development from those that do not (Fig. 4). Several chromosomes have an increased number of copy variations. Among them, chromosome 6 was altered in 16.6% of the contraction cases when compared to a 5% of the embryos that do not collapse ($p = 0.03$). Other chromosomes displayed similar alterations in blastocyst that collapse when compared to the group that did not, such as chromosome number 1 (9.6 vs 6%; $p = 0.045$), number 2 (11.4 vs 3.3%; $p = 0.028$), number 8 (11.3 vs 4.1%, respectively; $p = 0.03$) and number

Fig. 2 Chromosomal complements from 662 aneuploid top-quality embryos after PGT-A testing. Three complements have been taken into account: chromosome gain or loss and partial alterations (not distinguished between gain/loss). Percentage for each complement has been

calculated considering all aneuploid sample. Chromosomes 21 and 22 showed to be more sensitive to gain a copy when altered, whilst numbers 15 and 16 commonly lack one of their copies

Fig. 3 Number of contractions in high-quality embryos with different genetic complement. Euploid embryos (234/896) display the lowest number of episodes of contractions. Analysis on the aneuploid categories showed that embryos affected with monosomies (116/896) present less number of contractions when comparing with embryos affected with trisomies (136/896) or complex aneuploidies (410/896) (1.23 vs 1.53 and 1.40; $p < 0.05$), but no difference was observed when comparing the latter groups

19 (13.5 vs 5%; $p = 0.038$). In these chromosomes, the most common alteration in embryos exhibiting contractions is the gain of one copy.

Clinical outcome of high-quality euploid embryos

In Table 2, a univariate analysis was carried out to compare clinical outcomes for embryos which did not collapse to those

that presented the event during blastulation. A reduction in the pregnancy rate was observed in blastocysts that presented contractions when comparing them with the non-contracting group (47.6 vs 78.5%, respectively; OR, 0.22; CI 0.16–0.48; $p = 0.035$). Similar findings were obtained when comparing the clinical pregnancy rate, being lower in the group with embryos presenting contractions (40.0 vs 59.0%, respectively; OR, 0.36; CI 0.17–0.88; $p = 0.045$). To evaluate the effect of

Fig. 4 Chromosomal complements in 662 top-quality aneuploid embryos considering contraction presence. Two different profiles have been generated differentiating embryos without contractions (A, $n = 256$) from those presenting contraction events (B, $n = 406$). Descriptive analysis

shows a higher number of chromosome alterations (gains and losses) in embryos displaying contractions. (In brackets, total of embryos with an alteration in each chromosome; percentage of chromosomal abnormalities represented over 100%, considering the entire group)

Table 2 Univariate analysis of clinical outcomes of patients, who had a single embryo transfer in a frozen cycle with euploid embryos that presented contractions compared with those whose embryos did not

	No contractions (136 embryos; 91 embryos replaced)	Presence of contractions (98 embryos; 53 embryos replaced)	<i>p</i> value
Pregnancy rate	78.5 (74.9–83.1)	47.6 (38.1–69.8)	0.035
Clinical pregnancy rate	59.0 (45.1–62.1)	40 (38.1–49.9)	0.045
Clinical miscarriage rate	8.9 (5.5–10.2)	9.9 (5.1–13.3)	0.681
Ongoing pregnancy rate	49.9 (44.1–52.2)	31.2 (28.6–33.9)	0.069

five potential confounders, a logistic regression analysis for clinical pregnancy was conducted (Supplemental Table 2). Of the five variables, embryo contraction had a statistically significant effect on clinical pregnancy (OR 0.68 CI (0.63–0.73), $p = 0.013$). As expected, female age was not a significant factor affecting clinical pregnancy. This can be attributed to the fact that only euploid embryos were used for replacement.

Discussion

Choosing the embryo with the best implantation potential that has the right chromosomal content has become a feasible option with the introduction of PGT-A techniques such as NGS or aCGH at the blastocyst stage. One of the main drawbacks is the fact that this technique is invasive and may cause embryo trauma, which could hinder viability and thus implantation potential of a euploid embryo [8, 23]. Therefore, some algorithms based on embryo morphology or morphokinetics have been suggested to propose a non-invasive approach in identifying euploid embryo(s).

Euploidy rates of high-quality blastocysts are similar between day 5 or 6 biopsies

The present study examines embryo ploidy in day 5 and 6 blastocysts. Previous literature has suggested that embryos with slow development, and therefore reaching blastocyst on day 6 or 7, tend to be chromosomally abnormal [23–25]. Very few data have been collected regarding the relationship between standardised embryo quality grading and aneuploidy tested with the current technology (i.e. next-generation sequencing). A study comprising 500 blastocysts analysed via comparative genomic hybridization highlighted a correlation between aneuploidy and blastocysts morphology [26], but biopsy day was not considered in the analysis. Our current study illustrates that high-quality embryos displayed similar euploidy rates regardless of the biopsy day

present this event. Pregnancy rate and clinical pregnancy rate were reduced after replacement of euploid embryos that exhibited contractions during blastulation

(28.1% day 5 vs 22.3% day 6). However, authors have not investigated the euploidy/aneuploidy rate on poorer quality embryos, where the rates may be different when comparing day 5 or 6 developments.

Aneuploidies are common in embryos reaching blastocyst stage

As described in previous literature, the presence of aneuploidies in good quality embryos is not rare [27]. Although further studies confirmed that abnormal genetic complements lead to human embryo arrest [28, 29], additional studies revealed that a considerable proportion of aneuploid embryos are able to reach blastocyst stage with high morphological scores [26]. However, improvements in screening technologies have allowed the examination of the embryo's chromosome complement in much more detail, revealing higher levels of aneuploidy (up to 74.7%) in blastocysts derived from oocyte donors using aCGH [30]. These findings are concordant to the aneuploidy rate described in the present study (73.9%), but may be not representative of the whole population given the maternal age distribution of the sample (38.05 ± 2.9).

Next-generation sequencing technologies are the latest advance in comprehensive chromosome screening and the information obtained has increased over previous methods. Because of the level of detail provided by the technique, it is not surprising that the full extent of complete and partial aneuploidies, including mosaicism, can be detected more accurately. Our dataset highlights that complex aneuploidies, described as a combination of gains, losses and partial alterations affecting two or more chromosomes, can be the most common complement in high-quality embryos (61.5%), confirming previous findings that aneuploid embryos can develop further after genomic activation (day 3) even reaching blastocysts with high morphological scores. Our findings confirm that embryo morphology is clearly insufficient to describe the competence status of the blastocyst, which can be severely aneuploid regardless of its quality.

Embryo competence might be impaired by the presence of contractions in euploid embryos

Having studied the contraction behaviour in high-quality embryos, it is clear that embryo contraction is an event predominantly, but not exclusively, present in aneuploid embryos. The frequency of contractions seems to be strongly associated with the chromosomal complement. It is likely that alteration in epithelial permeability is a normal process for blastocyst development. Embryos may benefit from this event to enhance remodelling or repairing of the TE epithelium [16].

Given that our data show that aneuploid embryos exhibit a higher frequency of contractions when compared to euploid embryos (1.57 vs 0.6, respectively), we examined the premise that embryo contractions could compromise development and embryo health. Our data show that this event is associated with lower reproductive competence after frozen cycle transfer. The pregnancy rate falls from 78.5% to a 47.6% in transferred embryos when contractions are present. Similarly, the clinical pregnancy rate is affected in a way such that where embryos display the event, the percentage is reduced to 40% (compared to 59%). Following a multivariate logistic regression analysis considering other covariates, the clinical pregnancy rate continues to be reduced when transferring one euploid embryo that displayed contractions during its development. As highlighted previously, some of the embryos may still be mosaic and although being classified as euploid, this could be affecting the clinical pregnancy rate after transfer. Ongoing pregnancy rate was not different between groups, which may be due to the proportion of patients reaching that stage.

Differences in the number of copies of a chromosome underlie variations in gene expression. Interestingly, some of the blastocyst formation gene families (i.e. Na/K-ATPase pumps, adherens and gap or tight junctions) are located on the chromosomes that showed copy gain in embryos presenting with contractions (i.e. chromosomes 1, 6 and 19). Of all the aneuploid embryos, monosomies exclusively showed the lowest contraction rate. Trisomies and complex aneuploidies demonstrate a higher rate of this event happening, but no statistically significant difference was found between the groups. These findings suggest that embryo contraction, despite being a physiological feature during blastulation, is conditioned by the ploidy status of the embryo.

The effect of assisted hatching on the embryo contraction presence

One of the limitations of the study is the fact that all embryos were hatched on day 3 of development prior to biopsy. This procedure changes completely the dynamics of expansion and

our observations here only apply to the non-normative status of the zona pellucida after assisted hatching. Concordant to this, recent unpublished data from Esbert et al. [31] showed a statistical reduction in the presence of contractions in artificially hatched embryos (10.9%) when compared to those with an intact zona pellucida (16.7%; $p = 0.009$) [31]. Therefore, embryo contraction might be reduced, but not completely lost by the effect of assisted zona hatching.

It is clear that the chromosomal content plays an essential role in early embryogenesis and it seems to be tightly related to some features such as embryo contractions. Embryo surface variations, and other contraction pattern variables, might be early predictors of embryo competence. It may also help in describing embryo implantation potential which may result in higher success rates in ART cycles. This has not been analysed in the present study. The physiological purpose of embryo contractions is not clear, although the current study shows that their presence reduces embryo competence. Moving forward, further research could provide an answer whether contractions are a deleterious effect of aneuploidy or a mechanism to compensate chromosomal abnormalities. From a different perspective, it would be interesting to study the premise that contraction could be impacting mitotic chromosome segregation. The current study may be useful for further research in the field to describe the relationship and/or exchange of blastocoelic fluid with the media embryos are cultured in. Advances in genetic screening are probably opening a new era to the understanding of embryo ploidy and development.

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