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Reassessing the conventional fertilisation check: leveraging PGT-A to increase the number of transferrable embryos

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Structured abstract

Research question: Are some non-2PN zygotes normally fertilised, euploid and thus available for transfer?

Design: Retrospective cohort study on patient data from 1,214 PGT-A cycles a single private IVF clinic. 152 non-2PN embryos were assessed by PGT-A with parent of origin assessment and were compared to 4,822 2PN embryos assessed by PGT-A. Transfer outcomes of euploid embryos were then compared.

Results: Over 40% of the tested non-2PN embryos were euploid. The 152 non-2PN (88 ICSI; 64 IVF), embryos were 2 x 0PN, 50 x 1PN, 24 x 2.1PN, 65 x 3PN, 9 x 4PN and 2 x 5PN. Of 4,822 2PN embryos, 4,737 (98.2%) were diploid, 1.4% polyploid and 0.4% haploid. Embryos resulting from 1PN zygotes were more likely to be haploid. Embryos resulting from 3PN and 4PN zygotes were more likely to be polyploid ($p < 0.001$). 44/88 (50%) ICSI embryos were either haploid or polyploid; this was significantly higher than those derived from IVF (16/64, 25%, $p = 0.004$). Maternal origin of polyploidy was more common in ICSI cases ($p = 0.004$). 42% of the 90 diploid non-2PN embryos were also euploid, with diploid blastocysts from non-2PN zygotes being as likely to be euploid as those from diploid 2PN zygotes ($p = 0.10$). 12 euploid 1PN, 2.1PN and 3PN embryos have been transferred. Two ongoing pregnancies and four live births are reported.

Conclusions: This approach extends the use of PGT-A in identifying more embryos available for transfer, has the potential to increase cumulative pregnancy rates and questions the value of the fertilisation check.

Key words: abnormal fertilisation, embryo, euploid, pronuclei, zygote.

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Introduction

Despite decades of innovation, the principal limiting factor in both *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) continues to be the availability of sufficient numbers of suitable embryos for transfer (Capalbo et al., 2017; McLernon and Bhattacharya, 2023; Vaegter et al., 2017). This is especially pertinent for poor prognosis patients, where the most cited risk factor is advanced maternal age, and for low responders to controlled ovarian stimulation. Hence, while oocyte number is arguably the most significant predictor of cumulative live birth rate (McLernon and Bhattacharya, 2023), normal fertilisation is also essential and, accordingly, embryologists perform a "fertilisation check" prior to continued culture. This conventionally happens between 16 and 18 hours post insemination (in both IVF and ICSI) and fertilisation is deemed to be either successful, or not, by zygote morphology alone. At this check, the visible extrusion of the second polar body (PB) and the presence of two pronuclei (2PN) are the sole determinants of whether an embryo goes forward for continued culture and, thereafter, possible transfer (Capalbo et al., 2017).

Abnormal fertilisation may result in zygotes that are: a) morphologically apronuclear (0PN); b) have only one pronucleus (1PN); or c) are polypronuclear, having three, four or five pronuclei (3PN, 4PN, 5PN respectively). A final commonly observed phenomenon is a zygote that has two normally sized pronuclei, plus a smaller pronucleus (a micronucleus); these are termed "2.1PN" embryos. All of these abnormal types of fertilisation are observed in at least 10% of the zygotes produced by IVF or ICSI (according to Capalbo et al., 2017) although most clinics report 2PN rates of 65–80% per mature oocyte (ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine, 2017; Papale et al., 2012). It is however well established that zygotes assessed to be 2PN (diploid) may not be euploid (nor

indeed actually diploid) when genetically tested, with this a key rationale for PGT-A (reviewed in Griffin and Oğur, 2018; Victor et al., 2020).

Due to the unconfirmed fertilisation (and ploidy) status of non-2PN zygotes, and the potential risks that their transfer may carry such as miscarriage or molar pregnancy, abnormally fertilised embryos are not usually considered for clinical use and are routinely discarded. It has however been shown that, if left in culture, some of these zygotes can continue their development to the blastocyst stage, and that a proportion have the potential to result in live births (Capalbo et al., 2017; Kemper et al., 2023). As evidence suggests that the incidence of 1PN oocytes is 1–8% and of 3PN oocytes is 1–7% (ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine, 2017; Papale et al., 2012), the whole assisted reproduction community may be unnecessarily wasting viable embryos if a significant proportion of these are in fact diploid (and euploid).

Historically, all 3PN zygotes have been assumed to be triploid, and hence were not cultured further (Staessen and Van Steirteghem, 1997). It has however since been shown that some 3PN zygotes are actually diploid and that, when genetically tested, most of these are also euploid (Mizuta et al., 2023; Mutia et al., 2019, Capalbo et al. 2017). Similarly, whilst 1PN zygotes are usually haploid, the formation of morphologically normal blastocysts from these has been demonstrated since the early 1990s (Plachot and Crozet, 1992). A possible cause for this is asynchrony of PN formation. In some instances, a second PN forms between four and six hours after the fertilisation check (Staessen et al., 1993). 1PN morphology may also arise due to asynchrony of the formation of the nuclear envelope, resulting in either the failure to form the pronuclei envelope around one of the genomes, or that two prenatal

genomes are packed into a single envelope (Capalbo et al., 2017). In the late 1990s, the first evidence that blastocysts derived from 1PN zygotes could be successfully transferred and ultimately result in live births emerged (Barak et al., 1998; Gras and Trounson, 1999; Staessen and Van Steirteghem, 1997), and this has been confirmed since (Bradley et al., 2017; Capalbo et al., 2017; Dasig et al., 2004; Gras and Trounson, 1999; Gu et al., 2021; Henry et al., 2023; Hirata et al., 2020; Itoi et al., 2015; Li et al., 2020; Reichman et al., 2010; Si et al., 2019; Ussher et al., 2023; Xie et al., 2018).

2.1PN embryos are characterised by the presence of a small pronucleus alongside two normal pronuclei (Capalbo et al., 2017). These are not routinely considered for treatment due to the potential polyploidy risk involving extra genetic material. There is however now evidence that these too can be successfully cultured to the blastocyst stage. For example, Takahashi and colleagues identified 15 blastocysts that developed from 2.1PN zygotes and, although all of these were found to be diploid, they were predominantly aneuploid (Takahashi et al., 2022). Canon and colleagues subsequently reported a live birth and an ongoing pregnancy following the transfer of blastocysts that resulted from 2.1PN zygotes. The authors suggest that this type of embryo may result from failed cytokinesis, with some of one of the parental nuclei being driven into another envelope post fertilisation (Canon et al., 2023). Moreover, whilst other types of abnormal fertilisation events (0PN, 4PN, 5PN etc.) are far less common than those discussed above, live births have nonetheless also been achieved following the transfer of both 0PN (Destouni et al., 2018; Li et al., 2021; Liu et al., 2016; Paz et al., 2020) and 4PN-derived embryos (Bredbacka et al., 2023).

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To the best of our knowledge, no study has yet evaluated the proportion of different types of non-2PN embryos that are both diploid and euploid using PGT-A and the outcomes of the transfer of those that are deemed to be chromosomally normal. The objective of this study was therefore to establish the chromosomal constitution (including parent of origin) of a large cohort of zygotes that had been classified as abnormally fertilised through conventional fertilisation checks. In so doing, we confirmed if normal fertilisation had indeed occurred (despite evidence to the contrary) and assessed whether these embryos had potential for future transfer. The findings presented here are significant as they provide evidence to suggest that the applicability of PGT-A could be extended from an IVF embryo screening tool, to one that identifies embryos to be suitable for transfer that would not normally be considered.

Materials and methods

This study represents a retrospective cohort analysis undertaken using data derived from 1,214 PGTA cycles undertaken in a single UK center between April 2020 and January 2024. Embryos were cultured to the blastocyst stage in an Embryoscope (Vitrolife) time lapse incubator and a fertilisation check was undertaken 16 to 18 hours post insemination for both IVF and intracytoplasmic sperm injection (ICSI) cases. ICSI was proposed for patients who had male factor infertility or previous failed fertilisation from IVF. PGT-A was undertaken for all blastocysts, and parental swabs were provided for genotype analysis to confirm the parent of origin and ploidy status of the 152 embryos that showed deviations from 2PN (non-2PN), plus a further 4,822 embryos that were 2PN for comparative purposes. The use of patient data for this analysis was approved by the University of Kent Research

Ethics Advisory Group, approval number (CREAG114-07-23). Patients that used donor gametes were excluded from this study.

Assisted reproductive cycles

All women underwent ovarian stimulation of multiple follicle development using a GnRH antagonist protocol. Oocyte retrieval was scheduled for 36 to 38 hours following the administration of a human chorionic gonadotropin (hCG) trigger injection. Patients were assigned to either conventional IVF or ICSI according to semen parameters and their clinical history. Sperm was prepared using a density gradient, and for IVF, co-incubation with sperm occurred two hours post oocyte retrieval. For ICSI, oocytes were incubated for a minimum of two hours before being denuded using hyaluronidase media. A single sperm was selected based on morphology and was immobilised prior to injection. All embryos were cultured in continuous single culture medium (Vitrolife) pre-equilibrated the day before to 37°C in an atmosphere of 6% CO_2. For both IVF and ICSI cases, fertilisation was assessed 16-18 hours post insemination, with normal fertilisation being confirmed by the presence of two polar bodies (2PB) and 2PN (Ahuja et al., 1985). Zygotes exhibiting abnormal fertilisation were categorised as: apronuclear (0PN); monopronuclear (1PN); or polypronuclear, specifically, tri-pronuclear (3PN), tetrapronuclear (4PN), pentapronuclear (5PN) or 2.1PN (micro 3PN) as defined above. Embryos were subsequently assessed on days 5, 6 and 7 and those that developed to the blastocyst stage were graded in accordance with the ACE/NEQAS embryo grading guidelines (Balaban et al., 2011). To ascertain the ploidy status of the fully developed blastocysts, on either day 5 or day 6 of culture, trophectoderm (TE) biopsy was performed, with five to seven TE cells being removed and subsequently vitrified (Gorodeckaja et al., 2020; Thornhill et al., 2012).

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Aneuploidy parent of origin testing

To determine the parental origin of any chromosomal abnormalities in non-2PN embryos, parents provided buccal swabs, collected using DNA Genotek OCD-100 buccal swab kits provided by CooperSurgical, Inc. (Livingston, NJ). DNA extraction and analysis was performed on these samples and PGT-A was conducted by CooperSurgical, Inc. (Livingston, NJ) using the PGT-CompleteSM Test. Detection of genome-wide copy number variation (CNV) in non-2PN embryos, and the parental origin of these, were validated using control families with confirmed trio (parental/embryonic) relationships. Briefly, genomic DNA was isolated from maternal and paternal buccal swabs and processed with corresponding embryos. Next-generation sequencing (NGS) was used to collect low coverage sequence data across the respective genomes. The identification of CNVs and single nucleotide polymorphisms (SNPs) was performed in parallel. An initial karyotype was determined from CNV analysis of normalised read counts across all chromosomes. The ratio of sex chromosomes was used to identify potential male triploids (69;XXY) and all female euploids (46;XX) were further analysed. Genome-wide SNPs were filtered for depth and quality, and samples with greater than 200 SNPs included in validation. Identification of informative heterozygous SNPs and analysis of the allele ratios was used to determine PN status: euploids displaying an expected B allele frequency of 1:1, triploids 2:1, and haploids 1:0. Overlapping SNP data across the embryonic and parental samples was used to determine the inherited haplotypes and confirm maternal and paternal contribution. Euploid embryos (46;XX/XY) displayed balanced parental contribution in autosomes, while triploid (69;XXX/XXY) and haploid (23;X) embryos displayed the expected genome-wide

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imbalance. Confirmed cases from each group were used to construct a statistical model to predict non-2PN status of embryos.

Embryo vitrification and warming procedures

Blastocysts were vitrified using the Irvine Scientific® protocol after double-witnessing. Embryos were equilibrated in a M-199 HEPES buffered medium with gentamicin (7.5% dimethyl sulfoxide, 7.5% ethylene glycol, 20% dextran serum supplement (DSS)) for 12 minutes in a pre-labelled dish, before being moved to vitrification solution. The vitrification solution was again M-199 HEPES buffered medium with gentamicin, but this time containing 15% DMSO, 15% ethylene glycol, 0.5 M sucrose, 20% DSS. Cryotop® devices (Kitazato BioPharma Co. Ltd. Fuji, Shizuoka, Japan) were loaded with embryos and minimal freezing media and were then plunged into liquid nitrogen (-196°C). On the morning of embryo transfer, embryos were warmed by submerging the Cryotop® device in 37°C pre-equilibrated warming solution (Irvine Scientific). The embryos were then kept in warming solution of M-199 HEPES buffered medium with gentamicin (1 M sucrose, 20% DSS) for one minute, a solution that contained half the concentration of sucrose (0.5 M) for three minutes, and then a washing solution that contained no sucrose for five minutes before being moved to pre-equilibrated embryo culture media (Vitrolife) and incubated in an Embryoscope incubator (Vitrolife). Two hours following warming, blastocyst re-expansion was assessed.

Embryo transfer and the establishment of pregnancy

When embryos that were initially classified as abnormal were found to be euploid, 2PN embryos were given priority for transfer. If no 2PN embryos were available, then euploid

non-2PN embryos were considered for transfer. Endometrial preparation involved hormonal replacement therapy in 96% of the frozen embryo transfer cycles (FET). Cycles were initiated by the administration of 6-10mg of estradiol valerate (Progynova, Bayer) once the endometrial thickness was sufficient. Cyclogest 400mg (L.D. Collins and Co. Ltd) three times daily was provided for luteal support. On the morning of the embryo transfer, after obtaining consent from the patients, the selected embryos were warmed (see above), with the embryo transfer procedure performed within a window ranging from two to five hours post-warming procedure using an ultrasound-guided technique. Luteal support continued if pregnancy was confirmed, and an ultrasound scan was conducted at approximately eight weeks of gestation to confirm viability.

Data analysis

All data were analysed in R version 4.2.2 (R Core Team, 2022), using RStudio (RStudio Team, 2020). Proportion data were compared by chi-square goodness of fit or by Fisher's exact tests, with simulated *p* values used for comparisons of more than two groups. Where appropriate, *post hoc* testing was undertaken via Bonferroni-corrected pairwise comparisons to embryos derived from 2PN zygotes.

Results

Within the study period, 152 zygotes were identified at the fertilisation check stage as showing deviations from 2PN. Of these, two were 0PN, 50 were 1PN, 24 were 2.1PN, 65 were 3PN, nine were 4PN and two were 5PN (for representative images of these zygotes and the resulting blastocysts, see Figure I). Summary demographic data and the numbers of normal and abnormally fertilised oocytes are presented in Supplementary table 1. The rates

of second PB extrusion were higher in the 1PN and 2.1PN zygotes than those seen in the 3PN and 4PN zygotes (94.0% and 91.7% for 1PN and 2.1PN respectively, and 76.9% and 55.6% for 3PN and 4PN respectively) (Supplementary table 2). Overall rates of second polar body extrusion were also observed to be higher for euploid embryos when compared to their non-euploid counterparts (92.3% and 78.8% for euploid and non-euploid respectively). Given the low numbers of 0PN and 5PN zygotes, PB information in these groups cannot be meaningfully compared.

Most of these embryos were derived from ICSI (88/152, 57.9%), with the remainder generated via regular IVF (64/152, 42.1%). The frequency of abnormal fertilisation events did not differ between the two (Supplementary table 3: Fisher's exact test, p = 0.51), although there were proportionally more 2.1PN, 3PN, and 4PN zygotes from ICSI and more 1PN and 5PN from IVF.

Examination of routine PGT-A cases also permitted the analysis of a larger cohort (4,822) of 2PN embryos. Results show that almost all (4,737/4,822, 98.2%) the blastocysts derived from 2PN zygotes were diploid, with only a small fraction (85/4,822, 1.8%) shown to be either polyploid (1.4%) or haploid (0.4%) (Table 1). Results also indicated that the majority (93/152, 61%) of the 152 zygotes identified at the fertilisation check stage as non-2PN were in fact diploid, and hence were normally fertilised (Table 1). Analysis of these data indicated that the proportions of haploid and polyploid embryos differed between zygote groups (Fisher's exact test with simulated p values, p = 1e-07). Subsequent *post hoc* comparison of the embryos resulting from abnormally fertilised zygotes to those resulting from 2PN zygotes indicated that embryos resulting from 1PN zygotes were more likely to be haploid,

embryos resulting from 3PN zygotes were more likely to be polyploid as were embryos resulting from 4PN zygotes (all $p < 0.001$ in Bonferroni-corrected pairwise Fisher's exact tests). Other pairwise comparisons were non-significant ($p > 0.05$ in comparisons by Fisher's exact test with embryos derived from 2PN zygotes), an expected result given the limited numbers of embryos derived from 0PN, 2.1PN, 4PN and 5PN zygotes.

Interestingly, 44 of the 88 (50%) embryos derived from ICSI were either haploid or polyploid, a significantly higher proportion than that seen in the embryos derived from IVF where 16 of the 64 (25%) embryos were haploid or polyploid (Fisher's exact test, $p = 0.004$, Supplementary table 4). As parental cheek swabs had been obtained from the parents of these embryos, the parental origin of the haploidy and polyploidy could be assessed (Supplementary table 4). Analysis of these data indicated that a maternal origin of polyploidy was much more likely in zygotes derived from ICSI than from IVF (Fisher's exact test, $p = 0.004$).

PGT-A analysis also indicated that many (42%) of the 90 diploid embryos derived from abnormally fertilised zygotes were also euploid (Table 2). Analysis of these data indicated the proportions of euploid and aneuploid embryos did not differ between zygote groups (Fisher's exact test with simulated p values, $p = 0.10$). That is, these data indicate that diploid embryos from abnormally fertilised zygotes that develop to the blastocyst stage are as likely to be euploid as those from 2PN zygotes. Further to this, three of these euploid embryos did not extrude a second PB.

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Our PGT-A analysis also allowed the characterisation of aneuploidy type (Table 2). Analysis of the distribution of abnormality type, stratified as simple (≤ 2 chromosomes affected) or complex (> 2 chromosomes affected), indicated that the zygote groups did not differ in the patterns of aneuploidies that were detected (Fisher's exact test with simulated *p* values, $p = 0.12$).

At the time of writing, after obtaining consent, 12 of the euploid 1PN, 2.1PN and 3PN embryos had been transferred. The outcomes of these transfers can be seen in Table 3, with these data not further analysed given the limited number of transfers.

Discussion

This study focussed on the analysis of embryos that were assessed as abnormal at the fertilisation check, but that, when left in culture, completed development to the blastocyst stage. PGT-A testing of these embryos indicated that that most were diploid and therefore that normal fertilisation had occurred. Over 60% of the embryos identified as abnormal at the fertilisation check were in fact normally fertilised. Of these, over 40% were euploid and suitable for transfer. These results are broadly in line with other studies that have identified euploid embryos amongst those identified as abnormally fertilised (e.g.: (Canon et al., 2023; Capalbo et al., 2017; Lim and Lee, 2019; Mizuta et al., 2023; Mutia et al., 2019)). Given that three of the embryos identified as euploid in this study did not extrude a second polar body, both key elements of the fertilisation check are called into question

There are a number of potential explanations for the results we observe. The pseudo-excrusion of chromosomes when the second polar body is extruded for example, may be a

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potential explanation for the occurrence of diploid embryos with more than two pronuclei. Retention of chromosomes from the second polar body, even though the polar body itself is expelled, could result from a defect in the oocyte machinery, leading to a haploid set of chromosomes that should have been ejected remaining within the oocyte. In these cases, while syngamy may proceed normally, leading to the formation of a diploid zygote with a complete set of chromosomes, the additional set of chromosomes from the retained second polar body may not participate in syngamy but instead persist as an extra pronucleus. Over time, these additional chromosomes degrade or fail to contribute to embryo development. Further to this, abnormally fertilised embryos may also result from the retention of the second polar body, which is usually extruded during the final stages of meiosis. This would result in the zygote containing both its own pronucleus and the additional pronucleus from the retained polar body, potentially resulting in polyploidy.

Importantly, the overall rate of euploidy or aneuploidy seen here was not significantly different between embryos derived from abnormally fertilised zygotes and those derived from 2PN zygotes (Table 2). Abnormally fertilised zygotes that develop to the blastocyst stage and are diploid therefore had the same chance of being euploid as those developing from 2PN zygotes. These data therefore indicate that abnormally fertilised zygotes may be a valuable source of transferable embryos, particularly in cases where 2PN zygotes are absent or limited. The small sample size of 0PN, 4PN, and 5PN embryos is however a limiting factor in this study and as such generalised conclusions about these categories cannot be made. At the time of writing, the transfer of euploid embryos derived from 1PN, 2.1PN and 3PN zygotes have all resulted in healthy live births, with additional pregnancies ongoing. This further supports the view that healthy babies can result from the transfer of abnormally

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fertilised embryos, providing they are chromosomally normal. Many similar studies have focussed on one type of abnormal fertilisation; for example, Kobayashi and colleagues (2021) and Lui *et al*., (2016) focussed on 0PN embryos, whilst Li *et al*., (2020) concentrated on 1PN embryos. In contrast, despite the relatively small dataset presented here, we show that all types of abnormal fertilisation events can result in euploid embryos. As a result, we tentatively propose an order in which abnormally fertilised embryos should be considered for PGT-A testing and potential subsequent transfer. Specifically, we would advocate for the prioritisation of 1PN embryos, followed by 2.1PN, and then 3PN embryos, based on their diploid rates (Table 1).

Interestingly, PGT-A testing of different types of abnormally fertilised embryos showed that there was a large amount of variation within the different abnormality types. For example, some non-euploid 3PN embryos were triploid, whilst others were polyploid. Comparatively simple aneuploidies were also seen in the dataset; for example, whilst many of the non-euploid 1PN embryos were haploid, most were found to be aneuploid with abnormalities in two or fewer chromosomes. This study also found that the majority of the ICSI polyploidy was digynically derived, perhaps indicating that the oocyte chromosomal spindle could be negatively affected by this type of assisted reproductive technology (ART). The use of the latest sequencing technology also highlights that diploid embryos only having maternal or paternal chromosomes could be detected and eliminated as these could lead to pregnancy loss through ovarian teratomas or hydatidiform moles. It would be unwise therefore to transfer embryos derived from non-2PN zygotes without appropriate genetic testing. This suggests that there is a potential unexpected advantage of PGT-A. Specifically, critics of PGT-A argue that it can reduce the number of embryos available for transfer and hence the

cumulative live birth rates (Griffin and Oğur, 2018; Victor et al., 2020). These data however demonstrate that if PGT-A is used to screen embryos derived from abnormally fertilised zygotes, it is possible to make additional embryos available for transfer. If we assume that *c.*30% of embryos are non-2PN (ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine, 2017; Papale et al., 2012) and ~40% of these are euploid (results from this study) then, for the first time, PGT-A can improve cumulative pregnancy rates, formerly something it was considered unable to do. With mean reported numbers of blastocysts available as 2.44 for IVF and 2.11 for ICSI (Speyer et al., 2019), then a significant increase would mean more successful assisted reproduction for thousands of people.

In alignment with others (Capalbo et al., 2017; Doody, 2021), we therefore contend that a re-evaluation of the fertilisation check step in ART is warranted. The significant advances being made in the development and use of prediction algorithms in ART (Chapple et al., 2023; Fukunaga et al., 2020) and in the field of artificial intelligence, may further assist in optimal embryo assessment and selection (Shen et al., 2022).

The fertilisation check, when embryologists examine embryos for signs for successful fertilisation between 16- and 18-hours after oocyte and sperm co-incubation, or after ICSI is still a part of routine clinical practice in ART labs. It has been for over 40 years. Given the developments that have been made in the field throughout this time, a growing body of evidence suggests that “abnormal fertilisation” events may be inappropriately named. Now may therefore be the time to re-evaluate this standard practice across the sector, particularly when PGT-A is indicated.

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Data availability

The data underlying this article cannot be shared publicly due to privacy reasons.

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Conflict of interest

The authors have no conflicts of interest to declare in relation to this work.

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Legend to Figure

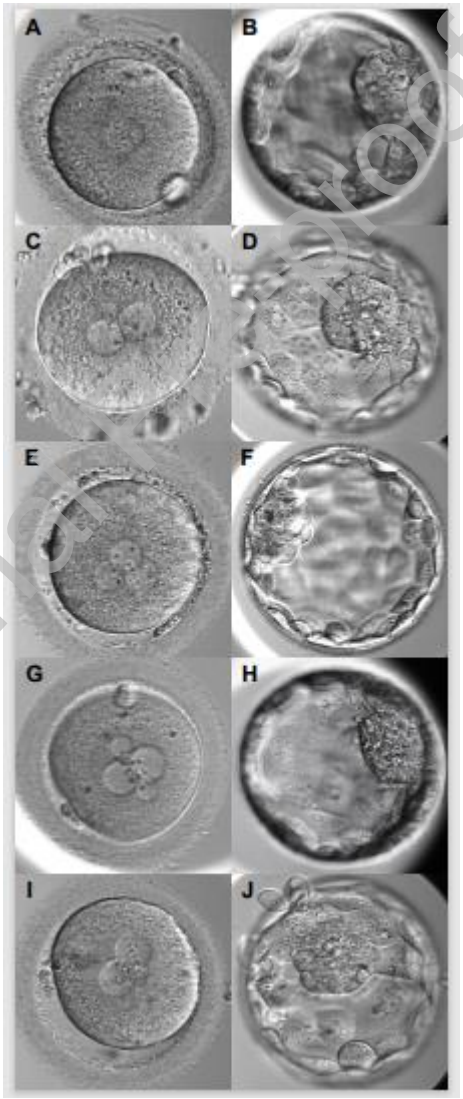

Figure I: Representative images of non-two pro-nuclei (PN) zygotes, and the resulting blastocysts after culture. A, C, E, G, and I are 1PN, 2.1PN, 3P N, 4PN and 5PN zygotes

respectively. The images to the right of each (B, D, F, H, J) are the corresponding resulting blastocysts.

Table 1: 152 embryos classified as 0PN, 1PN, 2.1PN, 3PN, 4PN and 5PN at the fertilisation check and tested by PGT-A. Those that were identified as diploid, haploid or polyploid with the number (and percentage) are shown. For comparison, the 4,822 embryos that were classified as 2PN were also assessed for ploidy status (established from routine screening) using PGT-A.

	Number of PGT-A tested embryos	Number (%) of diploid embryos	Number (%) of haploid embryos	Number (%) of polyploid embryos
0PN	2	1 (50%)	0 (0%)	1 (50%)
1PN	50	38 (76%)	10 (20%)	2 (4%)
2.1PN	24	24 (100%)	0 (0%)	0 (0%)
3PN	65	25 (38.5%)	7 (10.8%)	33 (50.8%)
4PN	9	3 (33%)	0 (0%)	6 (67%)
5PN	2	2 (100%)	0	0
Total non-2PN	**152**	**93 (61.2%)**	**17 (11.2%)**	**42 (27.6%)**
2PN	4,822	4,737 (98.2%)	17 (0.4%)	68 (1.4%)

Table 2: PGT-A results for the diploid embryos indicating the number (and percentage) that were euploid and aneuploid (including mosaics), and a comparison of the nature of the aneuploidies.

	Total number of diploid embryos	Number (%) euploid	Total number (%) aneuploid (including mosaics)	Fraction (%) of aneuploid embryos with ≤2 chromosomes involved	Fraction (%) of aneuploid embryos with >2 chromosomes involved
0PN	1	0 (0%)	1 (100%)	1/1 (100%)	0/1 (0%)
1PN	38	21 (55.3%)	17 (44.7%)	12/17 (70.6%)	5/17 (29.4%)
2.1PN	23	8 (34.8%)	15 (65.2%)	9/15 (60%)	6/15 (40%)
3PN	25	9 (36%)	16 (64%)	7/16 (43.8%)	9/16 (56.3%)
4PN	3	0 (0%)	3 (100%)	2/3 (66.7%)	1/3 (33.3%)
5PN	2	1 (50%)	1 (50%)	0/1 (0%)	1/1 (100%)
Total non-2PN	**92**	**39 (42.4%)**	**53 (57.6%)**	**31/53 (58.5%)**	**22/53 (41.5%)**
2PN	4,737	1,639 (34.6%)	3,098 (65.4%)	2,238/3,098 (72.2%)	860/3,098 (27.8%)

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Table 3: Outcomes of transferred embryos with number (percentage) at each stage. Maternal age is presented as mean ± standard deviation.

	1PN (maternal age 37.7 ± 5.8)	2.1 PN (maternal age 39 ± 3.5)	3PN (maternal age 37.5 ± 0.7)
Embryos transferred	6	3	3
Negative β-HCG test	4 (66.7%)	0 (0%)	1 (33.3%)
Biochemical pregnancy/miscarriage	0 (0%)	1 (33.3%)	0 (%)
Ongoing pregnancy	0 (0%)	1 (33.3%)	1 (33.3%)
Live birth	2 (33.3%)	1 (33.3%)	1 (33.3%)

Key Message

Aberrant fertilization occurs in many zygotes from IVF or ICSI, often leading to their discard. Some embryos initially deemed aberrant can develop to the blastocyst stage. PGT-A analysis helps identify viable embryos for transfer, increasing the number of available embryos.

Author Biography

Balsam Al Hashimi, an HCPC-registered clinical scientist, holds an MSc in clinical embryology from the University of Leeds. She serves as the Deputy Lab Manager and Lead Embryologist in Genetics at the London Women's Clinic (LWC) and is currently registered as a PhD student at the University College London.

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