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ARTICLE

Preimplantation genetic testing for aneuploidy 'rescues' poor-quality blastocysts and increases embryo availability for transfer: a 9-year single centre analysis

BIOGRAPHY

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KEY MESSAGE

Preimplantation genetic testing for aneuploidy allows identification of euploid poor-quality embryos (PQE), which can lead to live births and reduce miscarriage rates. Compared with untested PQE, transferring tested PQE may reduce the number of transfers needed per live birth. These findings support reconsidering the routine discard of morphologically poor embryos without genetic testing.

ABSTRACT

Research question: Does preimplantation genetic testing for aneuploidy (PGT-A) and the transfer of euploid poor-quality blastocysts (PQB) reduce the number of transfers needed to achieve live births compared with the transfer of their untested counterparts?

Design: Single-centre retrospective cohort study conducted between 2015 and 2024 (PGT-A blastocysts: $n = 7332$ from 2258 cycles; $n = 1344$). Transfer outcomes were analysed for a subset of 74 cycles involving tested PQB and compared with 192 cycles involving untested PQB during the same period.

Results: High-quality blastocysts (AA, AB, BA and BB) were most likely to be euploid ($P < 2.2e\text{-}16$), whereas PQB (CC, DC, CD and DD) were more likely to be aneuploid ($P < 2.2e\text{-}16$). Embryos that reached the blastocyst stage by day 5 had a higher likelihood of being euploid. Among transferred PQB, PGT-A did not significantly affect the pregnancy rate (33.3% versus 23.4%); however, the miscarriage rate was significantly lower in the PGT-A-tested group (13.6% versus 51.2%, $P = 0.003$). The number of live births was higher in the PGT-A group (26.4 % versus 11.1%, $P = 0.004$) and with the transfer of day-5 frozen blastocysts. Live births were observed from blastocysts with the poorest expansion and morphology scores. No significant differences were observed in gestational age or birth weight between the PGT-A and untested groups.

Conclusions: A clinically relevant proportion of PQB are euploid, PQB can result in live births and euploid transfer is associated with lower miscarriage rates. In combination, this suggests that PQB should not be routinely discarded, particularly if they are prior-tested using PGT-A.

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KEYWORDS

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INTRODUCTION

Despite decades of innovation in assisted reproductive technology (ART), one of the main predictors of a successful outcome remains to be embryo quality (*Gardner* et al., *2000*; *Van Den Abbeel* et al., *2013*; *Oron* et al., *2014*). Embryos are typically scored in accordance with the ACE/NEQAS grading system (*Association of Clinical Embryologists and UK NEQAS, 2017*), with high-quality blastocysts (HQB) (typically those graded as AA, AB, BA or BB) and lower quality blastocysts (LQB) (those graded as BC or CB), usually with an expansion score of 4, 5 or 6 being those that are usually considered for transfer (*Balaban* et al., *2011*; *Van Den Abbeel* et al., *2013*). Poor-quality blastocysts (PQB) (those graded as CC, CD, DC or DD) are often not considered for transfer and are commonly discarded owing to concerns about their potential for continued development and the increased likelihood of aneuploidy (*Kaartinen* et al., *2015*; *Chiappetta* et al., *2023*). Although discarding PQB may be an appropriate strategy when several HQB, LQB, or both, are available, this may not always be the case in practice. In some instances, only PQB may be recovered, or PQB may be obtained alongside limited numbers of HQB, LQB, or both. One way in which PQB are used clinically is in their transfer alongside other PQB, or with HQB or LQB to improve overall outcomes (*Oron* et al., *2014*; *Wintner* et al., *2017*; *Wang* et al., *2020*). Although double embryo transfer may improve success rates for poor prognosis patients it also increases the incidence of multiple births and is not a solution for all patients. The recovery of only PQB, or PQB alongside limited numbers of HQB, LQB, or both, is a situation more commonly seen in embryos retrieved from patients of advanced maternal age (AMA) (aged ≥35 years at the time of delivery [*Mehari* et al., *2020*]), reflecting the well-documented association between AMA, diminishing oocyte quality and higher rates of aneuploidy (*Franasiak* et al., *2014*; *Cimadomo* et al., *2018*; *Murphy* et al., *2019*). In these cases, PQB are more clinically valuable, and may represent the only option for treatment. With this in mind, and with emerging evidence challenging assumptions around their use (*Chiappetta* et al., *2023*), the practice of routinely discarding PQB is being re-evaluated. Indeed, it has been shown that a notable proportion of PQB can be euploid (*Munné* et al., *2005*; *Fragouli* et al., *2014*; *Viñals Gonzalez* et al., *2019*), and it has been established that those that implant can have the same potential for live births as their better-quality counterparts (*Kirillova* et al., *2020*). Further to this, despite their quality, a significant proportion of HQB and LQB are chromosomally abnormal (*Fragouli* et al., *2014*; Santamonkunrot *et al*., 2024).

Blastocyst trophectoderm biopsy, followed by vitrification and PGT-A analysis, aims to improve overall ART success rates by selecting euploid embryos for transfer, thereby enhancing implantation outcomes (*Al Hashimi* et al., *2025*) and reducing the risk of miscarriage (*Simopoulou* et al., *2021*). Although testing all available embryos in a cycle may offer a strategic advantage in that the embryo with the highest potential of resulting in a live birth may be identified earlier, and that the number of transfers required to achieve a live birth may be reduced, the PGT-A procedure itself has the potential to compromise embryo viability, thereby reducing cumulative birth rates. As such, PGT-A has been a much discussed, controversial topic in published research in the early 2000s, as reviewed recently by *Giuliano* et al. *(2023)* and *Morales (2024)*. Using PGT-A to test PQB, however, would not result in the potential loss of embryos that are transferable, as PQB are not routinely considered for transfer (*Griffin, 2022*); in fact, it has the potential to increase the overall number. The main objective of the present study was, therefore, to determine whether PGT-A and the subsequent transfer of euploid PQB led to improved outcomes compared with the transfer of their untested counterparts.

MATERIALS AND METHODS

This retrospective cohort study included 7332 PGT-A tested blastocysts obtained from 2258 cycles across 1344 patients at a single centre between 2015 and 2024. Transfer outcomes were analysed for a subset of 74 cycles involving tested PQB from 69 patients and compared with 192 cycles involving untested PQB from 180 patients during the same period. Nineteen patients had multiple transfers, four of these had three transfers, with the remainder having two. This analysis was approved by the University of Kent Research Ethics Advisory Group, which granted ethical approval for the use of anonymized patient data (approval number CREAG116-07-23; dated 5 December 2023).

Ovarian stimulation, fertilization and embryo culture

All patients underwent ovarian stimulation using urinary FSH (Menopur) (Ferring, Saint Prex, Switzerland), recombinant FSH (Gonal-F) (Merck, Rahway, NJ, USA), or both. To prevent premature luteinization, co-treatment with a gonadotrophin-releasing hormone antagonist (Fyremadel) (Ferring, Saint Prex, Switzerland) was administered. When the diameter of the leading follicle was greater than or equal to 18 mm, ovulation was triggered using either HCG (Ovitrelle) (Merck, Rahway, NJ, USA) or a gonadotrophin-releasing hormone agonist (Buserelin). Oocytes were retrieved 35–37 h later. Oocytes were fertilized either by IVF or intracytoplasmic sperm injection (ICSI). The latter was indicated because of either male factor infertility or previous fertilization failure with IVF. Fertilization was assessed 16–18 h after insemination, and embryos were cultured in an Embryoscope incubator (Vitrolife, Viby, Denmark). All embryos were subsequently incubated for up to 7 days, or until blastocyst formation and expansion, in continuous single culture medium (Vitrolife, Västra Frölunda, Sweden). Blastocysts were evaluated using the ACE/NEQAS grading system (*Association of Clinical Embryologists and UK NEQAS, 2017*), which scores the inner cell mass (ICM) and the trophectoderm on a scale from A to D. Examples of ICM and trophectoderm morphology are presented in Figure 1 and Figure 2, respectively. In the present study, HQB were defined as those graded AA, AB, BA or BB. Lower-quality blastocysts included those graded CB or BC, whereas PQB were those graded CC, DC, CD or DD.

Embryo biopsy and preimplantation genetic testing for aneuploidy

Preimplantation genetic testing for aneuploidy was conducted based on indications such as recurrent implantation failure, advanced maternal age (AMA) or prior miscarriage. Trophectoderm biopsy was carried out on selected blastocysts using the pulling and laser-assisted method (*McArthur* et al., *2005*), under sterile conditions and verified through double witnessing. Between five and seven cells were removed, washed and transferred into sterile Eppendorf tubes (CooperSurgical Inc, Trumbull, CT, USA), and PGT-A testing was carried out by CooperSurgical Inc. (Livingston, NJ, USA).

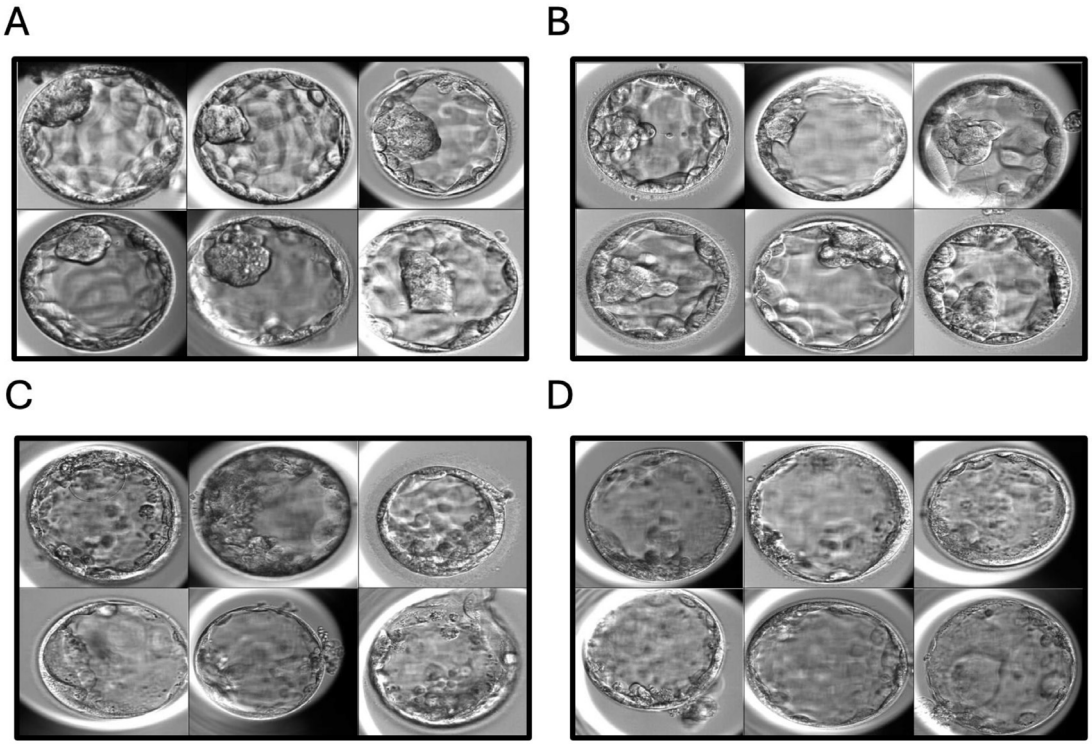

FIGURE 1 Representative examples of inner cell mass (ICM) blastocyst scoring. (A) grade A ICM (clearly defined and dense ICM composed of many tightly compacted cells forming a cohesive mass; (B) grade B ICM (moderately defined with loosely arranged cells that appear larger and less cohesive, occasionally showing distinct individual cells); (C) grade C ICM (poorly defined with few visible cells, either sparse or disorganised, often blending in with the trophectoderm); (D) grade D ICM (no identifiable inner cell mass or presence of degenerated or necrotic cells).

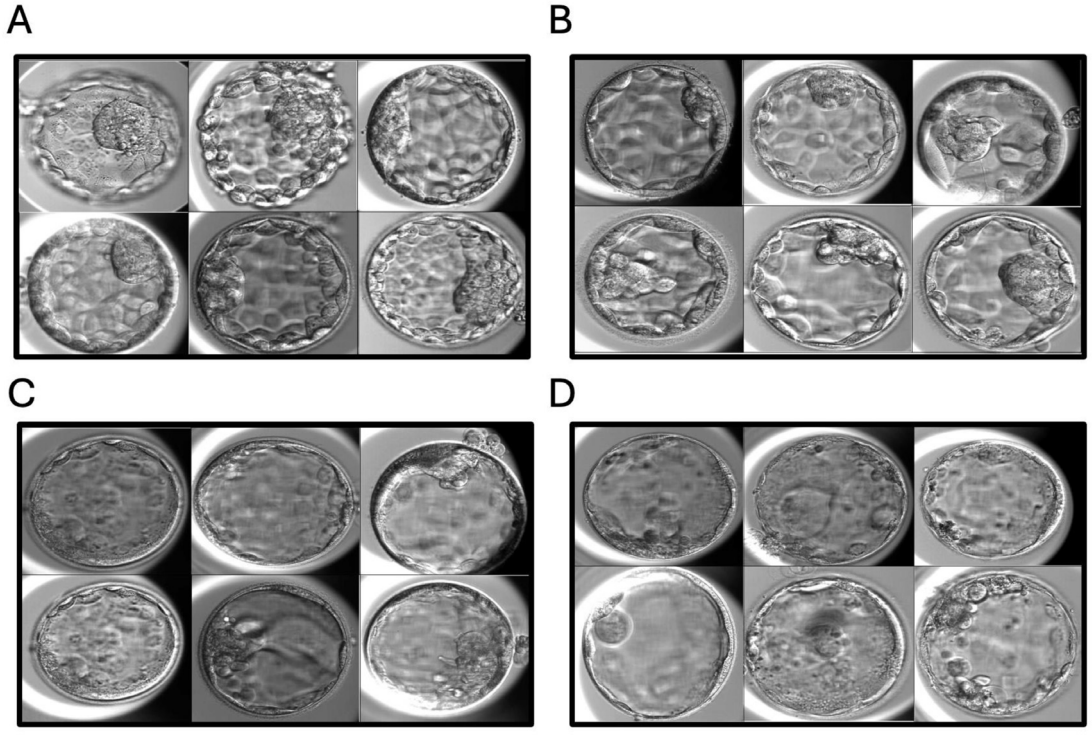

FIGURE 2 Representative examples of trophectoderm blastocyst scoring. (A) grade A trophectoderm (numerous small, uniform cells forming a cohesive and uninterrupted trophectoderm layer); (B) grade B trophectoderm (moderate number of cells with some gaps; the layer is partially continuous but not uniform; (C) grade C trophectoderm (contains a limited number of small cells interspersed with larger ones; the cell layer appears discontinuous; (D) grade D trophectoderm (few viable cells or predominantly degenerate cells).

Fifty-seven blastocysts (0.8%) were analysed using array comparative genomic hybridization, whereas the remaining 7275 blastocysts (99.2%) were tested using next-generation sequencing (NGS) platforms. Blastocysts analysed using array comparative genomic hybridization were tested before the introduction of NGS for this application. Embryos identified as euploid were considered for transfer.

Embryo vitrification and warming

All blastocysts included in this study were vitrified using the Irvine Scientific® (USA) protocol, as described in *Al Hashimi* et al. *(2024)*. Briefly, after double witnessing, blastocysts were transferred into a freezing dish and equilibrated for 12 min in a medium containing 20% dextran serum supplement and 7.5% each of ethylene glycol and dimethyl sulfoxide. Blastocysts were then transferred to a vitrification solution (Irvine Scientific, Santa Ana, CA, USA) containing 20% dextran serum supplement, 0.5 M sucrose and 15% each of ethylene glycol and dimethyl sulfoxide. Blastocysts were stored by plunging them into liquid nitrogen in Cryotop® devices (Kitazato BioPharma Co. Ltd., Fuji, Shizuoka, Japan) with minimal freezing media.

Two to five hours before transfer, a double witnessing step took place. The Cryotop® device containing the embryo was then submerged into pre-equilibrated (37°C) warming solution (Irvine Scientific, Santa Ana, CA, USA) for 1 min, followed by transfer into a solution with a lower concentration of sucrose for 3 min. After this, blastocysts were transferred to a solution containing no sucrose for 5 min. After warming, embryos were cultured in embryo culture media (Vitrolife, Västra Frölunda, Sweden) in an Embryoscope incubator (Vitrolife, Viby, Denmark). Embryo expansion, an indicator of viability, was assessed after a minimum of 2 h of incubation after warming.

Frozen embryo transfers and the establishment of pregnancy

A small proportion of patients (2%, $n = 4$) underwent frozen warmed embryo transfer (FET) as part of a natural cycle with luteal support using Cyclogest 400 mg (LD Collins and Co Ltd, Hemel Hemstead, Herts, UK). To compare transfer outcomes, a subset of 74 cycles involving tested PQB from 69 patients were compared with 192 cycles involving untested PQB from 180 patients during the same period. Nineteen patients had multiple transfers, four of these had three transfers, with the remainder having two. The remaining 98% underwent FET with hormone replacement therapy. For these patients, endometrial preparation began with a baseline scan conducted in the early follicular phase. After a normal scan result, patients commenced oestradiol valerate (Progynova) (Bayer, Leverkusen, Germany) at a dose of 6–10 mg/day to stimulate endometrial proliferation. Treatment with oestradiol continued for 10–17 days. Once an endometrial thickness of 7 mm or more was achieved, luteal support was initiated with progesterone pessaries (Cyclogest 400 mg) administered three times daily. After 5 days, a single embryo was warmed, cultured for a minimum of 2 h to ensure re-expansion and viability, and was transferred between 2–5 h after warming. Luteal support continued until pregnancy was biochemically confirmed (defined as a serum β-HCG level exceeding 100 mIU/mL measured 12 days after embryo transfer). Hormonal support was then maintained until at least 7–8 weeks of gestation, when a transvaginal ultrasound scan was conducted to confirm a clinically viable intrauterine pregnancy by the presence of a fetal pole and heartbeat. A miscarriage was defined as the spontaneous loss of a clinically confirmed pregnancy before 12 weeks of gestation.

Data analysis

Data analysis was undertaken in R version 4.2.2 (*R Core Team, 2022*), using RStudio (*RStudio Team, 2020*). Proportion data were compared using Fisher's exact tests. Simulated *P*-values were used for comparisons of more than two groups, with post-hoc testing, where appropriate, undertaken by pairwise Bonferroni corrected Fisher's exact tests. Quantitative variables were analysed using Kruskal–Wallis tests, with Bonferroni corrected Dunn tests used for post-hoc testing. Unless otherwise noted, data are reported as mean ± SD. For PGT-A tested blastocysts, stepwise logistic regression was used to determine if euploidy was associated with blastocyst quality assessment (HQB, LQB or PQB), maternal age, ART method (ICSI/IVF), day of blastocyst freezing or pre-vitrification expansion. For the transferred PQB, the outcomes of live birth rate, gestation length and birth weight were compared between PGT-A and untested blastocysts. Stepwise logistic regression was then used to determine if live birth rate from the transfer of PQB was affected by PGT-A, ART method (ICSI/IVF), oocyte age at collection, maternal age at transfer, day of embryo transfer or expansion.

RESULTS

Of the 7332 PGT-A tested blastocysts considered, no difference was observed between blastocyst quality groups in their likelihood of coming from ICSI or IVF (TABLE 1). Analysis of age at embryo collection did, however, indicate differences between all groups ($P < 2.2e-16$), with most PQB coming from the older patients (TABLE 1). As expected, the PQB were also less expanded ($P = 0.00001$) and, on average, required more time to reach the blastocyst stage ($P = 0.00001$) (TABLE 1).

A PGT-A result was obtained from 7199 blastocysts, with a total of 133 tests failing to return a result. Blastocyst quality was found to affect the likelihood of test success, with PGT-A testing of 1.5% of HQB (57/3846), 1.8% of LQB (40/2185) and 2.8% of PQBs (36/1301) not resulting in a determination of ploidy status ($P = 0.01$, Fisher's exact test). Analysis of the PGT-A results indicates that HQB were most likely to be euploid ($P < 2.2e-16$), with the PQB more likely to be aneuploid ($P < 2.2e-16$) (TABLE 2). Stepwise logistic regression of these data indicated that HQB were most likely to be euploid (LQB, adjusted OR 0.62, 95% CI 0.55 to 0.70, and PQB, OR 0.38, 95% CI 0.32 to 0.45). Blastocysts were also more likely to be euploid if they came from younger patients (adjusted OR 0.89, 95% CI 0.88 to 0.90), or had expanded more (adjusted OR 1.38, 95% CI 1.26 to 1.51). In contrast, blastocysts were less likely to be euploid if they had been derived from IVF rather than ICSI (adjusted OR 0.72, 95% CI 0.64 to 0.80) and day 6 (adjusted OR 0.70, 95% CI 0.62 to 0.78) and day-7 blastocysts (adjusted OR 0.46, 95% CI 0.34 to 0.61) were also less likely to be euploid. That is, embryos reaching the blastocyst stage on day 5 were more likely to be euploid. The proportion of euploid blastocysts across different age and quality groups is presented in TABLE 3 (a representation of these data is also shown in Supplementary Figure 1).

To date, 74 FET cycles of PGT-A-tested PQB have been initiated. At the time of writing, remaining euploid blastocysts were either still in storage or were discarded after the patient completed their family. A comparator group of 192 FET cycles of untested PQB were identified, with baseline parameters presented in TABLE 4. Analysis of these data indicated that the

TABLE 1 BASELINE CHARACTERISTICS FOR PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY BLASTOCYSTS

Characteristics	HQB (*n* = 3846)	LQB (*n* = 2185)	PQB (*n* = 1301)	*P*-value
ICSI/IVF	67.2 (2586)/32.8 (1260)	65.9 (1441)/34.1 (744)	67.9 (884)/32.1 (417)	*P* = 0.43
Age at oocyte retrieval, years	36.8 ± 4.3[a]	37.3 ± 4.2[b]	38.2 ± 4.2[c]	Kruskal–Wallis chi-squared = 121.5, df = 2, *P* < 2.2e-16
Day of embryo freezing				*P* = 1e-5
5	66.8 (2568)	37.6 (821)	16.0 (208)	
6	32.2 (1240)	56.5 (1234)	67.6 (879)	
7	1.0 (38)	5.9 (130)	16.4 (214)	
Pre-vitrification expansion, grade				*P* = 1e-5
2	0.0 (0)	0.0 (1)	0.5 (6)	
3	7.7 (298)	10.6 (232)	15.5 (202)	
4	81.0 (3114)	81.0 (1769)	78.9 (1026)	
5	6.6 (253)	4.3 (95)	1.8 (24)	
6	4.7 (181)	4.0 (88)	3.3 (43)	

Data presented as % (*n*) or mean ± SD.

The proportion of blastocysts derived from intracytoplasmic sperm injection was analysed by Fisher's exact test. Age at oocyte retrieval was compared by Kruskal–Wallis test, with post-hoc testing by Bonferroni corrected Dunn test.

[a–c] Results of post-hoc analyses; groups with different letters are significantly different (adjusted *P*- values as follows, HQB versus LQB, *P* = 6e-6; HQB versus PQB, *P* = 3e-27; LQE versus PQE, *P* = 6e-10).

Df, degrees of freedom; HQB, high quality blastocyst (typically those graded as AA, AB, BA or BB; LQB, lower quality blastocyst (typically graded as BC or CB); PQB, poor-quality blastocysts (typically graded as CC, CD, DC or DD).

TABLE 2 PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY RESULTS FOR TESTED BLASTOCYSTS

Outcome	HQB	LQB	PQB	*P*-value
Total with PGT-A result, *n*	3789	2145	1265	–
Euploid, % (*n*)	46.0 (1743)[a]	31.1 (668)[b]	18.5 (234)[c]	<2.2e-16
Aneuploid	41.4 (1568)[a]	57.6 (1235)[b]	70.4 (890)[c]	<2.2e-16
High-level mosaic	6.0 (229)	5.7 (123)	6.6 (83)	0.61
Low-level mosaic	6.6 (249)[a]	5.5 (119)[a,b]	4.6 (58)[b]	0.02

Data presented as % (*n*).

Fisher's exact tests were used to compare the proportion of blastocysts of each category.

[a–c] Results of post-hoc Bonferroni corrected pairwise Fisher's exact tests analyses; groups that share the same letter do not significantly differ (*P* > 0.05), whereas those with different letters are significantly different (*P* < 0.05). Precise *P*-values for the post-hoc tests are presented in Supplementary Table 2.

HQB, high-quality blastocyst (typically those graded as AA, AB, BA or BB); LQB, lower quality blastocyst (typically graded as BC or CB; PQB, poor-quality blastocysts (typically graded as CC, CD, DC or DD).

tested PQB were derived from older individuals and transferred into older patients. Blastocyst quality was also marginally higher in the untested PQB than in those that had undergone PGT-A (*P* = 0.002) (TABLE 4).

In both the tested and untested groups, a small proportion of embryos did not survive warming, but this did not differ between groups (with tested PQB survival at 97.3%, *n* = 72/74 and untested PQB survival at 97.9%, *n* = 188/192, *P* = 0.67, Fisher's exact test). Embryos that survived warming proceeded to transfer, with no difference observed between the pregnancy rates seen in the tested and untested PQB (TABLE 5), although one positive beta-HCG test was a biochemical pregnancy from the tested group and one implanted embryo in each of the groups had to be terminated as they were ectopic. Among the remaining blastocysts, miscarriage rates were higher in the untested PQB group compared with those that underwent PGT-A testing (*P* = 0.003), whereas live birth rates were significantly higher in the PGT-A tested group (*P* = 0.004) (TABLE 5). In contrast, no significant differences were observed between the two groups in gestational age or birth weight of the resulting offspring (TABLE 5). As some patients underwent multiple transfers, and information on their reason(s) for treatment were not considered here, these data were also analysed with all multiple transfers excluded. This did not change any of the results (data not shown).

Stepwise logistic regression of these data indicated that an increase in live birth rate was associated with PGT-A testing (adjusted OR 5.03, 95% CI 2.19 to 12.00) and with the transfer of day-5 blastocysts (adjusted OR 0.22, 95% CI 0.10 to 0.48 for day-6 blastocysts, with no live births observed from the transfer of day-7 blastocysts). In this analysis, embryo age (individual age at egg collection), maternal age at transfer, method of ART (ICSI or IVF), expansion grade and blastocyst quality were not found to be significant predictors of live birth (Supplementary Table 1). As above, re-analysis of these data with all multiple transfers excluded does not change these results (analysis not shown). Importantly, however, of the 40 reported live births, multiple resulted from the transfer of blastocysts with expansion

TABLE 3 DISTRIBUTION OF EUPLOID BLASTOCYSTS ACROSS AGE GROUPS AND BLASTOCYST QUALITY

Blastocyst quality	Total embryos tested	18–34 years (*n* = 1745), % (*n*)	35–37 years (*n* = 1712), % (*n*)	38–40 years (*n* = 2013), % (*n*)	41–43 years (*n* = 1526), % (*n*)	44–47 years (*n* = 203), % (*n*)
AA	533	62 (104/168)	63 (79/125)	50 (76/153)	47 (37/79)	0 (0/8)
AB	438	67 (73/109)	50 (58/116)	54 (69/128)	29 (22/77)	13 (1/8)
BA	218	71 (52/73)	62 (31/50)	46 (28/61)	28 (8/29)	20 (1/5)
BB	2600	59 (390/663)	50 (329/657)	35 (248/709)	26 (134/524)	9 (4/47)
BC	1821	41 (177/429)	38 (165/430)	29 (145/497)	16 (63/402)	3 (2/63)
CB	324	55 (44/80)	44 (34/77)	28 (28/101)	16 (10/61)	2 (1/5)
CC	783	30 (43/144)	25 (41/162)	20 (44/221)	10 (23/220)	8 (3/36)
CD	166	21 (6/29)	28 (11/39)	14 (6/42)	14 (7/49)	0 (0/7)
DC	77	42 (5/12)	33 (6/18)	29 (7/24)	6 (1/17)	17 (1/6)
DD	239	21 (8/38)	26 (10/38)	8 (6/77)	9 (6/68)	0 (0/18)

Data presented as percentages of euploid blastocysts (number of euploid blastocysts/total number). Quality graded according to the ACE/NEQAS system.

TABLE 4 BASELINE CHARACTERISTICS FOR PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY TESTED AND UNTESTED POOR-QUALITY BLASTOCYSTS WARMED FOR TRANSFER

Characteristics	PGT-A tested (*n* = 74)	Untested (*n* = 192)	*P*-value
Age of individual at oocyte retrieval, years	37.7 ± 3.9	34.9 ± 5.7	Kruskal–Wallis chi-squared = 13.47, df = 1, *P* = 0.0002
ICSI/IVF	66.2 (49)/33.8 (25)	68.8 (132)/31.3 (60)	*P* = 0.77
Blastocyst quality (CC, CD, DC, DD)	71.6 (53)/6.8 (5)/5.4% (4)/16.2 (12)	86.5 (166)/7.8 (15)/2.1 (4)/3.6 (7)	*P* = 0.002
Expansion, grade			*P* = 0.13
2	1.4 (1)	2.1 (4)	
3	6.8 (5)	17.2 (33)	
4	83.8 (62)	76.0 (146)	
5	5.4 (4)	3.6 (7)	
6	2.7 (2)	1.0 (2)	
Day of blastocyst freezing			*P* = 0.36
5	31.1 (23)	34.4 (66)	
6	54.1 (40)	56.8 (109)	
7	14.9 (11)	8.9 (17)	
Maternal age at transfer, years	39.6 ± 3.8	38.1 ± 5.9	Kruskal–Wallis chi-squared = 4.47, df = 1, *P* = 0.03

Values presented as %(*n*) or mean ± SD of the mean. Proportions were analysed by Fisher's exact test. Continuous data were analysed by Kruskal–Wallis test.

Df, degrees of freedom; ICSI, intracytoplasmic sperm injection.

scores of 2 or 3 and morphology scores of CC, with several resulting from blastocysts with morphology scores of CD and DD.

DISCUSSION

The data indicate that a clinically relevant proportion of PQB (18.5%) are euploid, and that both PGT-A-tested and untested PQB can result in live births. These findings are in concordance with *Fragouli et al. (2014)*, who concluded that selecting embryos for transfer based on their morphological appearance alone did not exclude aneuploid embryos. Like others (*Majumdar et al., 2017*), our data show that HQB were most likely to be euploid and that PQB were most likely to be aneuploid. As expected, blastocysts were more likely to be euploid if they came from younger patients (*Matorras et al., 2024*) or if they had expanded more (*Huang et al., 2019*). In contrast, blastocysts were less likely to be euploid if they had been derived from IVF rather than ICSI and day-6 and day-7 blastocysts were also less likely to be euploid; that is, embryos reaching the blastocyst stage on day-5 were more likely to be euploid (*Cimadomo et al., 2022*; *Corti et al., 2022*). A high proportion of PQB in the present study were derived from older patients, with tested PQB both

TABLE 5 OUTCOMES FOR TRANSFERRED PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY TESTED AND UNTESTED POOR-QUALITY BLASTOCYSTS

Outcome	PGT-A tested (*n* = 72)	Untested (*n* = 188)	Analysis
Positive pregnancy test[a]	33.3 (24/72)	23.4 (44/188)	*P* = 0.12
Miscarriage rate[b]	13.6 (3/22)	51.2 (22/43)	*P* = 0.003
Live birth rate	26.4 (19/72)	11.1 (21/188)	*P* = 0.004
Gestation period, weeks	37.8 ± 2.3	38.4 ± 1.1	Kruskal–Wallis chi-squared = 0.17, df = 1, *P* = 0.68
Birth weight, kg	3.2 ± 0.5	3.4 ± 0.6	Kruskal–Wallis chi-squared = 0.63565, df = 1, *P* = 0.43

Values presented as % (*n*) or mean ± SD of the mean.

Proportions were analysed by Fisher's exact test. Continuous data were compared by Kruskal–Wallis test.

[a] Serum HCG >100 mIU/ml.

[b] The spontaneous loss of a clinically confirmed pregnancy before 12 weeks of gestation.

Df, degrees of freedom; PGT-A, preimplantation genetic testing for aneuploidy.

derived from, and transferred into, older individuals. This reflects the well-established association between advancing maternal age and increased rates of aneuploidy, primarily owing to meiotic errors during oogenesis (*Matorras* et al., *2024*). In addition, age-related declines in mitochondrial function and overall oocyte competence are thought to contribute to the reduced developmental potential and blastocyst quality observed in this population (*Cimadomo* et al., *2022*).

When considering transfer outcomes, we found that the pregnancy rates between tested and untested PQB were similar. This implies that the tested and untested PQB are implanting at similar rates. In contrast, we found that the miscarriage rate was higher in the untested PQB group, and that the live birth rate was higher in the PGT-A tested group, despite the greater age of the tested group. The differences in the miscarriage rate resulting from the transfer of tested PQB concurs with *Munné* et al. *(2005)* who suggest that testing for aneuploidy reduces the risk of miscarriage in recurrent miscarriage patients. We do, however, note that the proportion of recurrent miscarriages or recurrent implantation failure has not been compared between groups here, and this could be important for outcomes.

Importantly, comparison of transfer outcomes indicates that PGT-A has the potential to reduce the number of transfers required to achieve live births. Here, based on the live birth rates observed, an average of 9.0 transfers were required per live birth for the untested PQB, whereas for tested PQB, only 3.8 transfers were needed. Given that only 18.5% of PGT-A tested PQB in the present larger sample were euploid, this implies that an average of 20.5 PQB would need to be tested to identify the 3.8 transferred euploid blastocysts that resulted in each live birth. That is, the live birth rate per transfer is higher for PGT-A tested PQB (one per 3.8 transfers for tested versus 1 per 9.0 for untested); however, the live birth rate per developed embryo is higher for untested PQB (one per nine embryos for untested versus an estimated one per 20.5 for tested PQB). Given that the euploidy rate decreases with maternal age (**TABLE 3**), the difference between these two strategies will become more extreme in older patients. In an example in which the use of poor-quality embryos (albeit differently defined), was analysed by ART cycle, a 2.6% increase in the cycles that resulted in the incidence of one or more live births was observed (*Cimadomo* et al., *2019*). Although an analysis by cycle has not been conducted here, the number of PQB transfers that we estimate would be required per live birth suggest that a similarly modest increase in the number of cycles that would that result in the incidence of one or more live births would be expected here.

In conclusion, given the low overall success rates from untested PQB and the effect of unsuccessful transfers on patients and their families, PGT-A is likely to be of great interest to patients wishing to limit the number of transfers that they undergo and may be of particular value to individuals or couples with limited options. These data also provide a valuable new perspective in the ongoing debate (*Simopoulou* et al., *2021*; *Griffin, 2022*; *Giuliano* et al., *2023*; *Morales, 2024*) about the role and value of PGT-A testing. Taken together with recent evidence that suggests that PGT-A can be useful in screening embryos derived from abnormally fertilized oocytes (*Al Hashimi* et al., *2025*), our results indicate that testing PQB can increase the total number of transferrable blastocysts by recovering those that would otherwise be discarded.

UNCITED REFERENCES

Xi et al., 2022

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2025.105208.

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