

Introduction
Pre-implantation genetic testing for aneuploidy (PGT-A) was developed as a method to select embryos for transfer in in-vitro fertilization (IVF). Embryologists typically select embryos based on morphology; however, many women still do not achieve a pregnancy even after 'good quality' embryo transfers. PGT-A allows the screening of an embryo's genetic makeup before implantation, effectively allowing clinicians to not transfer aneuploid embryos.

In Singapore, strict rules are set in place, and only women who have subfertility and fulfil one of the following clinical criteria are eligible for PGT-A: (1) (i) 35 years and above, (ii) suffered two or more recurrent implantation failures (RIF), or (iii) experienced two or more pregnancy losses (RPL)

The clinical benefits of PGT-A in RPL patients, particularly young RPL patients, remain uncertain. Whether couples with unexplained RPL have higher embryo aneuploidy rates remains equivocal. Its improvement may be greater with advancing age. (2,3)

PGT-A is associated with its own risks and ethical challenges, such as high costs, difficulties in culturing embryos to the blastocyst biopsy stage, potential embryo damage or loss, and embryo wastage. (4) Newer research has shown that PGT-A is associated with decreased cumulative live birth rate (CLBR) amongst all patients who had blastocysts aged < 40. (5) Another of PGT -A 's limitations lie in the difficulty of interpreting a mosaic result. Even for a mosaic PGT-A result, the embryo can still be euploid, aneuploid, mosaic for both an euploid and aneuploid cell line, or mosaic for 2 or more different abnormal cell lines. (5) These difficulties could compound ethical considerations such as disposing viable embryos or transferring embryos with undetected issues.

In addition to the challenges of performing and interpreting PGT-A, causes of RPL can be multifactorial, ranging beyond aneuploidy. PGT -A as an intervention purely to exclude aneuploidy may in reality not be as beneficial to patients with RPL.

Aim
In light of this, our clinical audit aimed to explore the value of PGT-A in RPL patients in Singapore's largest women's Hospital.

Methodology
This is a retrospective audit of patients seen within our local setting in KK Women's and Children's Hospital's (KKWCH) IVF centre (KKIVF). All blastocysts had a single trophectoderm biopsy for PGT-A on Day 5 or 6 of embryo culture. Date of patients who underwent PGT-A at KKIVF Centre in our centre from 2017 to 2024 was extracted from our hospital's IVFKKIVF's database and de-identified. Data was analysed with SPSS ver.23. No ethical board review was required.

Results
Between 2017 and 2024, 124 patients underwent PGT-A at KKIVFin KKWCH centre.. 60 of these patients had RPL as their primary indication for PGT-A. A total of 24 patients with RPL had their biopsies cancelled and their embryos discarded. When stratified according to age, 18/49 (37%) of patients aged below 40 and 6/11 (55%) of patients aged 40 and above had their PGT-A biopsies cancelled. Amongst all RPL patients who underwent PGT-A, a total of 132 embryos were biopsied, of which 47%(n=62) were euploid and 53% (n=69) were aneuploid. In women less than 40 years old who had at least one embryo biopsied for PGT-A, Only 19% (6/31) of patients, who had at least one embryo biopsied, of RPL patients aged below 40 had an euploid-only culture results from their PGT-A embryo biopsy. 77% (24/31) had at least one aneuploid embryo detected by PGT-A.

However, , for patients aged 40 years and above who had embryo biopsied, 0% of RPLnone of the patients, who had embryo biopsied, had euploid-only culture results. All of the patients in this groupm had at least one aneuploid embryo detected by PGT-A. Interestingly, 80% of RPL patients aged 40 years and above (4/5) had aneuploid-only culture results.

These results are further summarized in the tables attached below.

Table 1: PGT-A Data on Patients with Recurrent Pregnancy Loss (RPL) from 2017 to 2024 in KKWCH

Total no. of patients eligible for PGT-A	(N=60)
No. of patients with biopsy cancelled	24 (40%)
Reasons for cancellation	
Abnormal/Poor development	12
Failed fertilisation (No 2PN)	3
Poor blastocyst outcome	1
ICSI not performed	1
Low number of embryos	2
Patient's or doctor's request	3
Others/not stated	2
No. of patients with biopsy done	36 (60%)
Total no. embryo biopsied	132
*Euploid	62 (47%)
*Aneuploid / Abnormal	69 (52%)
*Missing data	1 (1%)

Table 2: Embryo results for patients who underwent PGT-A for RPL stratified according to age

Total no. of patients who had embryos suitable for biopsy	Age <40 years old (n=31)	Age ≥ 40 years old (n=5)	Total (n= 36)
Euploid embryos only	6 (19%)	0 (0%)	6
Aneuploid embryos only	4 (13%)	4 (80%)	8
Mix of euploid and aneuploid embryos	20 (65%)	1 (20%)	21
Missing data	1 (3%)	0 (0%)	1

Discussion

From our study results, the cancellation rate of PGT-A for patients < 40 years old is 37%. The most common reason for PGT-A biopsy cancellation was poor embryo development. This high rate of cancellation could be because only patients with subfertility and RPL were recruited for PGT-A in our centre. For all RPL patients with biopsiable embryos, about 20% of these patients had euploid- only culture results. The low incidence rate of aneuploidy and high rate of cancellation might suggest that the value of PGT-A performed as screening for aneuploidy in young RPL patients might be overestimated. Patients should be thoroughly counselled on the high chance of non-conclusive biopsy result outcomes before proceeding with the cycle.

For RPL patients < 40 years old who had blastocysts available for biopsy, 13% were found to have aneuploidy- only culture results. Compared to RPL patients ≥ 40 years old, 80% of patients had aneuploidy-only culture results. Even though our study cohort size is small, these findings are in agreement with the conclusions of other larger scale studies suggesting that there is a high risk of aneuploidy among women ≥ 40 years old, making PGT-A a logical option for them.

When counselling young patients <40 years old with RPL, it is important to highlight that approximately 20% of these patients who undergo PGT-A may have only normal euploid embryos. Disadvantages of PGT-A such as requiring a freeze-all procedure post oocyte pick up, possible embryo demise before blastulation, potential embryo damage during biopsy and subsequent false positive results cannot be understated.

There are many contributory causes of RPL, and while aneuploidy is common, it should not be overemphasized. As a result, IVF with or without PGT-A should not be the standard treatment for young RPL patients. Using IVF with PGT-A solely to screen for aneuploidy might not be beneficial for this select group of patients.

Conclusion

Risk of PGT-A should be considered more seriously before routine use of PGT-A to screen for aneuploidy in the RPL population. This is especially important for young couples with RPL. Although RPL and subfertility can co-occur in the same patient, clinicians should be aware of the indications for the treatment prescribed, selecting only the most appropriate one available.