Pregnancy Outcomes of Pre-implantation Genetic Testing for Aneuploidy (PGT-A) Among Women of Advanced Maternal Age at the Center for Advanced Reproductive Medicine and Infertility: A Retrospective Cohort Study

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Abstract

Background: The benefits of preimplantation genetic testing for aneuploidy (PGT-A) in the advanced maternal age group are unclear.

Objective: This study aims to determine whether PGT-A improves pregnancy outcomes.

Methods: This is a retrospective cohort study of PGT-A outcomes using next generation sequencing for advanced maternal age women undergoing IVF at CARMI from May 2017 to May 2021. Women were grouped by age: those 35-39 and those 40 and above. Pregnancy rate (PR), live birth rate (LBR), and miscarriage rate (MR) were computed per transfer and per cycle and compared with women who underwent single day-5 frozen transfer of a morphologically chosen embryo.

Results: Overall euploid blastocyst rate was 38.5%: 16.9% for 40 and above and 47.6% for 35-39 group. There were no transfers in 41.4% due to absence of a euploid embryo. PR and LBR per embryo transfer were higher in the PGT-A versus the non-PGT-A group (61.9% vs 24.1% p=<0.001 and 42.9% vs 19% p=<0.001). By age, the findings were similar: higher PR and LBR per-embryo transfer in PGT-A versus non-PGT-A in the 35-39 group (58.4% and 29%, p=0.006 and 42.9% vs 22.6%, p<0.001 respectively) and 40 and above (71.4% vs 18.5%, p<0.001 and 53.6% vs 14.8%, p<0.001 respectively). MR was increased in the PGT-A versus non-PGT-A group, but this may be due to the small number of events in the population.

Conclusion: The study suggests an increase in PR and LBR per embryo transfer in advanced maternal age women undergoing PGT-A. A larger sample size is needed to validate the results.

Key words: in-vitro fertilization, aneuploidy, advanced maternal age

Introduction

Advanced maternal age, that is, maternal age 35 years old and above[1], is strongly associated with an increased number of abnormal oocytes due to segregation errors that happen during meiosis. When these abnormal oocytes are fertilized, aneuploid embryos are produced. Aneuploidy is defined as a deviation from the normal number of chromosomes, that is, the occurrence of one or more extra or a missing chromosome leading to an unbalanced complement of chromosomes.[2] It is the most common anomaly found in embryos obtained from in vitro fertilization (IVF)[3] leading to poor pregnancy outcomes, including higher miscarriage rates and increased risk of fetal chromosomal abnormalities and congenital defects.[4,5] The aneuploidy rate in women younger than 35 years old is less than 40%, whereas it doubles to 80% in women older than 44 years of age.[6] Aneuploid embryos are known to have

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poor implantation potential.[7] Miscarriage rates are increased 2-fold for those aged 35-39 and 2.4-fold for women aged 40 and above, and these rates are attributed to the higher embryo aneuploidy in these older age groups as compared to those less than 35 years of age.[8]

The quest for improving outcomes of IVF cycles has led to the application of add-ons in clinical practice. Choosing the best embryo for transfer can be challenging, as the tradition of choosing based on embryo morphology alone is not consistent and it does not accurately predict pregnancy outcomes.[9,10] Embryo grading by morphology is done by assigning a grade which describes the overall appearance in terms of cell symmetry and the extent of fragmentation.[11] For the day 5 embryo, the blastocyst rating includes the grading of the inner cell mass, the trophectoderm, as well as the degree of expansion of the blastocyst cavity using a system proposed by Gardner and Schoolcraft.[12]

Good embryo morphology also does not necessarily equate to embryo euploidy.[13] Preimplantation genetic testing for aneuploidy (PGT-A) is one of the add-ons available, and it is employed as a screening tool for choosing euploid embryos for transfer. This has the potential to increase live birth rate, reduce miscarriage rate, and shorten time to pregnancy.[14] PGT-A permits the biopsy of preimplantation embryos in order to eliminate the aneuploid embryos. Therefore, only the euploid embryos are selected for transfer. In theory, the selection of euploid or normal embryos, particularly for advanced maternal age women, allows exclusion of the aneuploid embryos in a group where aneuploidy is notably high. PGT-A, in this sense, offers a mechanism to screen for embryonic competency prior to transfer.[15,16,17]

Preimplantation genetic screening (PGS) was first used in 1990 to detect X-chromosome linked diseases in preimplantation embryos.[18] In 1993, geneticists started doing polar body biopsy and cleavage stage (day 3 embryos) biopsy. The biopsied specimen were analyzed by single-cell analysis using fluorescence in-situ hybridization (FISH). Polar body biopsy could only assess maternal genetic information. Cleavage stage biopsy, on the other hand, involved extraction of 1-2 cells (estimated 30% of 8 cells) of the day 3 embryo. This allowed the assessment of only 7-9 chromosomes. This was known as preimplantation genetic diagnosis for aneuploidy screening version 1.0.[19] Randomized controlled trials[20,21] and meta-analysis[22] however showed that preimplantation genetic diagnosis version 1.0 negatively affected the ongoing pregnancy rate and live birth rate in advanced maternal age women. Some problems identified with the technology included the examination of only a few number of chromosomes, the ineffectiveness of the FISH procedure, and the level of mosaicism in day 3 cleavage stage embryos.[23] Since then, significant improvements in the technology included the use of comprehensive chromosome screening which allowed the examination of all the 24 chromosomes. With the upgrade and incorporation of comprehensive chromosomal screening in 2008, the technology became known as preimplantation genetic diagnosis version 2.0.[24] The comprehensive chromosome screening was accomplished using the following genetic platforms: single nucleotide polymorphism microarrays, array comparative genomic hybridization, quantitative polymerase chain reaction, and next generation sequencing. Among the comprehensive chromosome screening tests, next generation sequencing is currently considered the gold standard for genetic analysis as it screens more information, is sensitive and specific, and is less costly as compared to the other platforms.[25,26,27,28] The shift from the binary euploid-aneuploid classification and the addition of the mosaic classification upgraded the technology to the preimplantation genetic diagnosis version 3.0. It is now known officially as preimplantation genetic testing for aneuploidy or PGT-A, particularly when preimplantation genetic diagnosis is done to improve pregnancy rates by weeding out the aneuploid embryos for transfer.[29]

In the 30 years that PGT-A technology has been available, its use remains controversial because it has inconsistently and inconclusively shown improvement in pregnancy outcomes. Despite its very wide usage worldwide, there is no strong body of evidence that currently support its use. The UK Human Fertilisation and Embryology Authority (HFEA) gave PGT-A for day 5 embryos a "red traffic light" guidance because randomized controlled trials did not show that it was effective at improving the chances of having a baby for most patients with infertility. It is also an added expense to an already

costly IVF procedure.[30] There are reports suggesting that any form of biopsy of the embryo may affect its development.[31] Errors in biopsy technique and interpretation may also lead to false positive outcomes, which may lead to the disposal of good and viable embryos that can otherwise lead to a healthy live birth.[31] On the other hand, a recent review of 190,010 cycles performed between the years 2016-2018 showed that the live birth rate per embryo transferred and per treatment cycle were higher for the PGT-A versus the non-PGT-A cycles and reduced the number of transfers per live birth for women over 40 years of age.[17] This was also supported by a large randomized multi-center trial that showed an increase in the ongoing pregnancy rate on a per embryo transfer basis in women 35-40 years old.[19] This is in contrast to a recent publication that showed no improvement in pregnancy outcomes in older women who underwent PGT-A.[32] As evidence is conflicting, the authors would like to add to the accumulating data regarding this technology from their set of patients. They aimed to determine if PGT-A improves outcomes for women with advanced maternal age.

The Center for Advanced Reproductive Medicine and Infertility, St. Luke's Medical Center-Global City, Philippines, (CARMI) is the first IVF center in the Philippines that used PGT-A. There is currently no Philippine data that describes the outcome of its use in advanced maternal age women. It is important to determine whether this technology has benefit for this population undergoing IVF in terms of pregnancy rate, miscarriage rate, and live birth rate. As an add-on, PGT-A is offered to patients who undergo IVF in the center, particularly to those with a history of recurrent pregnancy loss or recurrent implantation failure, those with advanced maternal age, those with a known parental chromosomal abnormality and those with severe male factor infertility and/or surgically retrieved sperm.

Aims and Objectives

This study aimed to answer the question of whether PGT-A improves pregnancy rate and live birth rate and decreases miscarriage rate in women with advanced maternal age undergoing IVF. It also aimed to determine the euploidy rate of blasts of women with advanced maternal age, as there is no Philippine data about this. Data from this research can provide center-based statistics and add to the accruing body of information worldwide. The data that acquired herein may also be used while counseling this subgroup of women about the pregnancy outcomes with the use of this technology.

Methods

This is a retrospective cohort analysis. Following the institutional ethics review board and University high-risk ethics approval, a review of the CARMI database was done. Women aged 35 years old and above who underwent IVF with PGT-A and subsequent frozen embryo transfer in CARMI from May 1, 2017, which was the time when PGT-A was first used in the center, until May 31, 2021 were included in the study. Women from the same age group who underwent IVF with single embryo transfer in a frozen cycle were included as the control group.

Cycles where sperm were surgically retrieved and those with severe male factor infertility were excluded. Those with a history of known parental chromosomal abnormality, recurrent pregnancy loss and recurrent implantation failure, and women with known uterine abnormalities (uterine congenital malformation, untreated uterine septum, or intrauterine adhesions) were excluded as well. Women with a prior history of pregnancy with a congenital or heredofamilial anomaly were also not included. Lastly, women who underwent double or multiple embryo transfers and those who underwent day 3 transfers or fresh embryo transfers were excluded from the study.

The remaining couples were screened and divided into two groups: the first group included those with day 5 embryos (blastocysts) that underwent PGT-A using next generation sequencing with subsequent single frozen embryo transfer and the second group included the advanced maternal age women that underwent non-PGT-A IVF cycles who underwent single frozen embryo transfer of a day 5 morphologically-chosen embryo. The 2 groups were further subdivided into two age groups, namely, a group that included women aged 35-39 years old and another group which included those 40 years old and above.

Stimulation and Transfer Protocol

The patients underwent ovarian stimulation using the antagonist protocol, which is the preferred and standard protocol used in the center. The stimulation cycle was monitored with serial ultrasound tests with or without estradiol monitoring. A transvaginal oocyte retrieval was performed 36 hours after injection of a trigger shot. The mature eggs (metaphase II oocytes) that were collected were fertilized using intracytoplasmic sperm injection at the 40th hour. The embryos were cultured for 5 days from the day of retrieval (blastocyst stage). For those in the control group, vitrification of blastocysts was done on day 5. For those in the PGT-A arm, the zona pellucida was breached to allow the trophoectoderm cells to herniate, after which, four to eight trophectoderm cells were biopsied by the CARMI embryologists and the embryos were subsequently vitrified. The specimen were sent to Thailand (Next Generation Genomics Co., Ltd. in Bangkok) for comprehensive chromosomal screening. The samples were then processed using a next generation sequencing assay (Next Generation Genomix, Thailand) following standard protocols and manufacturer's instructions. Prediction of ploidy was performed using a software algorithm, and the results were sent back to CARMI after analysis. Selection of a euploid blastocyst was made for transfer on a subsequent cycle, one embryo at a time. Frozen embryos were transferred on day 5 of a natural or hormone replacement cycle. Luteal phase support was in the form of oral and vaginal progesterone. Quantitative serum Beta-hCG results were determined 2 weeks after the embryo transfer.

Statistics

The sample size was calculated based on the comparison of the live birth rate between PGT-A and non-PGT-A embryo transfer. Assuming that the live birth rate for PGT-A and non-PGT-A are 40.3% and 11.0%, respectively based on the study by Sacchi et al[44], with an alpha error of 5%, power of 80%, and a one-tailed alternative hypothesis, the sample size calculated was 27 per group, for a total of 54 for two groups. For the comparison between the two age groups, the final sample size required was 108.

Statistical analysis was done using Statistical Package for the Social Science database (SPSS version 24) (SPSS, Chicago, IL, USA). A comparison of pregnancy rate, live birth rate per embryo transfer and per treatment cycle, and miscarriage rate of those that underwent PGT-A versus non-PGT-A, compared by age-group tier was done. Pregnancy rate is defined as those that had a positive Beta-hCG result (Beta-hCG > 5 mIU/mL). Clinical pregnancy is defined as those that had a gestational sac as documented by an ultrasound. Miscarriage refers to clinical pregnancies that were lost before the 20th week of gestation. Live births are those pregnancies that reached past 20 weeks age of gestation and subsequently delivered a live infant. These outcomes were analyzed using chi-square test. Stratified analysis was done using the age group tier as the stratification variable. Odds ratio with the 95% confidence interval for the euploidy rate was calculated. The level of significance was set at alpha 0.05.

Results

The Center for Advanced Reproductive Medicine started performing embryo biopsies with PGT-A using next generation sequencing in May 2017. From this time to May 2021, a total of 220 women aged 35 and above underwent PGT-A. During this 4 year period, six couples were excluded because the sperm used was surgically retrieved, and another 25 were excluded due to histories of recurrent pregnancy loss or recurrent implantation failure. Two couples were excluded because they were known carriers of chromosomal anomalies. Fifty-two couples had double embryo transfers and were excluded from the study. For the PGT-A arm, there was a total of 162 cycles performed among 135 couples that were included. From this group, 96 cycles were performed in those aged 35-39, and 66 cycles were for those aged 40 and above.

The control group consisted of 54 single embryo frozen transfer cycles of morphologically chosen day 5 embryos in women 35 years old and above. Of these, 31 women were aged 35-39, and 23 were aged 40 and above (Table 1). The comparison of the distribution of ages of the women within the PGT-A group and non-PGT-A group was similar, and was not statistically significant. It was noted

that there was a marked difference in the number of cycles in the PGT-A group compared to that of the non-PGT-A / control group.

Among those that underwent IVF, 29.6% had combined male and female factor as cause for infertility in both the PGT-A and non-PGT-A groups (Table 2). Among the female factors, purely advanced maternal age was present in 48.1% and 37% in the PGT-A and non-PGT-A group respectively. Anovulatory dysfunction was the most common female factor in this advanced maternal age patients (32.1% and 35.21% in the PGT-A group and the non-PGT-A group, respectively) (Table 3). Other infertility factors documented were tubal factors, endometriosis, and a combination of these female factors.

Table 1. Number of IVF cycles in the PGT-A group (Group 1) and the non-PGT-A group (Group 2), divided into age tiers 35-39 and 40 and above

		PGT-A (n=162)	Non-PGT-A (n= 54)	
		n (%)	n (%)	p value
Age group	35 – 39	96 (59.3%)	31 (57.4%)	0.811
	40 and above	66 (40.7%)	23 (42.6%)	

Table 2. Indication for IVF, female factor and combined male and female factor.

	PGT-A (n=162) n (%)	Non-PGT-A (n= 54) n (%)	p value
Female factor only	114 (70.4%)	38 (70.4%)	1
Male and female factor	48 (29.6%)	16 (29.6%)	

Table 3. Specific breakdown of female factor infertility: advanced maternal age and other female factors in addition to advanced maternal age

		Female factor infertility	
		PGT-A (n=162)	Non-PGT-A (n= 54)
		n (%)	n (%)
Specific female factor	Advanced maternal age only	78 (48.1%)	20 (37%)
	Tubal factor	18 (11.1%)	4 (7.4%)
	Anovulation	42 (25.9%)	12 (22.2%)
	Endometriosis	14 (8.6%)	9 (16.7%)
	Tubal factor and anovulation	4 (2.5%)	5 (9.3%)
	Tubal factor and endometriosis	0	2 (3.7%)
	Anovulation and endometriosis	6 (3.7%)	2 (3.7%)

For the PGT-A arm, there was a mean of 3.22 ± 2.48 blastocysts per patient, whereas there was only a mean of 2.74 ± 1.76 blastocysts per patient in those that did not undergo PGT-A. The total number of embryos per cycle in those that underwent PGT-A was higher, however, the difference was not statistically significant (p = 0.121) as presented in Table 4.

Overall, combining the patients in the PGT-A and non-PGT-A groups and comparing the number of blastocysts between age tier, there was a significantly higher number of blastocysts in the younger age group versus the older age group (3.45 ± 2.346 versus 2.61 ± 2.219, p = 0.008) as seen in Table 5. Segregating treatment and control groups into age tiers, a clinically higher number of blastocysts per cycle was seen in the 35-39 age group versus the older age group in the PGT-A group (3.5 ± 2.462 versus 2.82 ± 2.468, p = 0.086). This was not deemed statistically significant. The non-PGT-A group however showed a statistically higher number of blastocysts in the younger age group compared to those 40 years old and above (3.29 ± 1.979 versus 2.00 ± 1.087, p = 0.003) as seen in Table 5. In the older tier control group, 10 of the 23 patients had only 1 blastocyst from the stimulation cycle, whereas only 4 of the 66 in the 35-39 age group had only 1 blastocyst.

The overall euploidy rate was computed to be 38.5% (95% CI 34.3 – 42.9) in women 35 years and older (Table 6). Breaking this down according to age group, those aged 35 to 39 had a euploidy rate of 47.6% whereas those aged 40 and above had a euploidy rate of only16.9%, an expected significant finding (p < 0.001) (Table 7).

Among those that underwent PGT-A, 67 of 162 cycles (41.4%) yielded only aneuploid embryos or mosaic embryos. For these cycles, no euploid embryos were available for transfer. No transfers occurred in 62.1% in the older age group versus 27.1% in those less than 40 years old, which was a statistically significant finding (p < 0.001) as seen in Table 8. This can be directly correlated to the lower number of blastocysts per patient in the older age group. In the 40 and above age tier, 20 cycles did not have an embryo transfer because their lone embryo was aneuploid. This is compared to only 10 cycles which had 1 (aneuploid) embryo in the 35-39 age group.

Table 4. Mean number of blastocysts per cycle, in the PGT-A group versus non-PGT-A group

	PGT-A (n=162) Mean ± std. Deviation	Non-PGT-A (n=54) Mean ± std. Deviation	p score
Number of blastocysts per cycle	3.22 ± 2.48	2.74 ± 1.76	0.121

Table 5. Comparison of number of blastocysts by age between combined PGT-A and non-PGT-A groups, PGT-A group only and non-PGT-A group only.

	Age group				p value
	35-39		40 and above		
	n	Mean ± std. deviation	n	Mean ± std. deviation	
PGT-A and non-PGT-A group	127	3.45 ± 2.346	89	2.61 ± 2.219	0.008
Number of blastocysts in the PGT-A group	96	3.5 ± 2.462	66	2.82 ± 2.468	0.086
Number of blastocysts in the non-PGT-A group	31	3.29 ± 1.979	23	2.00 ± 1.087	0.003

Table 9 shows that once a transfer of a euploid embryo is made, the pregnancy rate, miscarriage rate and live birth rate were not statistically significant when comparing between the 35-39 versus the 40 and above age group.

Table 10 shows that there is a trend toward a higher pregnancy rate per cycle in those that underwent PGT-A compared to those that did not undergo PGT-A, however, this was not statistically significant (40.1% vs 25.9% respectively, p = 0.061). Pregnancy rate per embryo transfer however showed that there is a clinical and statistical improvement in the pregnancy rate in those that underwent PGT-A (61.9% vs 24.1%, p <0.001). Clinical pregnancy rate

Table 6. Euploidy rate of embryos.

	Number of Embryos	Number Normal	Rate (95% CI)
Euploidy rate	510	193	38.5% (34.3 – 42.9)

Table 7. Euploid rate of embryos that underwent PGT-A, by age group.

	35-39			40 and above			p value
	Number of Embryos	Number of Euploid Embryos	Euploidy Rate	Number of Embryos	Number of Euploid Embryos	Euploidy Rate	
Euploidy rate	319	152	47.60%	182	41	16.90%	<0.001

Table 8. Number of cycles with and without embryo transfer due to the availability of euploid embryos for transfer in the PGT-A group, stratified by age group

	Age group		p value
	35 - 39 (n=96)	40 & above (n=66)	
	n (%)	n (%)	
With embryo transfer	70 (72.9%)	25 (37.9%)	<0.001
No embryo transfer	26 (27.1%)	41 (62.1%)	<0.001

Table 9. Pregnancy outcomes of euploid embryos, compared by age group where total number of euploid embryos transferred for the 35-39 year old age group = 77 and total number of euploid embryos transferred for the 40 and above group = 28

	Age group		p value
	35 - 39	40 and above	
	n (%)	n (%)	
Pregnancy rate	45 (58.40%)	20 (71.40%)	0.226
Biochemical pregnancy rate	11 (14.30%)	2 (7.10%)	0.294
Miscarriage rate	4 (5.20%)	3 (10.70%)	0.294
Live birth rate	30 (39.00%)	15 (53.60%)	0.294

was 32.1% versus 24.1% per cycle (p < 0.266) and 49.6% versus 22.4% (<0.001) per embryo transfer, with a statistically significant increase in the PGT-A group, but this statistical difference was only seen on a per transfer basis. In this study, miscarriage rate per embryo transfer was significantly higher at 6.7% for the PGT-A group versus 3.4% in the control group (p < 0.001), but the number of miscarriages was overall few (7 in the PGT-A group and 2 in the non-PGT-A group). Live birth rate per embryo transfer was noted to be significantly higher in the PGT-A group compared to that of the control group (42.9% vs 19%, p < 0.001). The live birth rate per cycle was not statistically significant between the two groups (27.8% vs 20.40%, p = 0.207) as shown in Table 10.

The authors examined the pregnancy outcomes of the PGT-A group and non-PGT-A group and compared them according to age stratification, and a similar trend was seen. Table 11 shows that in the 35-39 age group, the pregnancy rate was higher in the PGT-A group, but was found to only be statistically significant in the per embryo transfer comparison between the PGT-A versus non-PGT-A groups (58.4% versus 29% respectively, p = 0.006). The higher live birth rate was also seen in the comparison of the 2 groups, where a statistically improved live birth rate was seen in those that underwent PGT-A, but only in the per embryo transfer comparison (42.9% vs 22.60%, p = <0.001). In the 40 and above age group, Table 12 shows clinically higher rates in pregnancy, clinical pregnancy, and live birth in those that underwent PGT-A. However, only statistically improved rates were seen in the per embryo transfer comparisons, where pregnancy rate was 71.4% versus 18.5% (p < 0.001). Clinical pregnancy rate was 64.3% versus 18.5% (p < 0.001), and live birth rate was 53.6% versus 14.8% (p < 0.001) in the PGT-A versus non-PGT-A groups, respectively. The finding of higher miscarriage rate in the PGT-A group was seen in both age stratifications, and clinically significant in the per embryo transfer comparison (6.7% versus 3.20% p < 0.001 in the 35-39 age group, and 10.7% versus 3.7% p < 0.001 in the 40 and above age group). Overall, the number of miscarriages was small in this study.

Discussion

The results of this study, although preliminary due to its limited sample size, suggest that preimplantation genetic testing for aneuploidy improves pregnancy rate and live pregnancy rate per embryo transfer in women of advanced maternal age undergoing in vitro fertilization in CARMI. Miscarriage rate was noted to be higher in the PGT-A

Table 10. Pregnancy rates (positive BhCG test), clinical pregnancy rate (with gestational sac documented sonologically) miscarriage rate and live birth rate per cycle and per embryo transfer (where total number of cycles in the PGT-A group = 162, total number of embryo transfers in the PGT-A group = 105, total number of cycles in the non-PGT-A group = 54 and total number of embryo transfers in the non-PGT-A group = 58)

	Treatment		p value
	PGT-A	non-PGT-A group	
	n (%)	n (%)	
Pregnancy rate per cycle	65 (40.10%)	14 (25.9%)	0.061
Pregnancy rate per ET	65 (61.9%)	14 (24.1%)	<0.001
Clinical pregnancy rate per cycle	52 (32.1%)	13 (24.1)	<0.266
Clinical pregnancy rate per ET	52 (49.6%)	13 (22.4)	<0.001
Miscarriage rate per cycle	7 (4.30%)	2 (3.70%)	0.207
Miscarriage rate per ET	7 (6.70%)	2 (3.4%)	<0.001
Live birth rate per cycle	45 (27.8%)	11 (20.40%)	0.207
Live birth rate per ET	45 (42.9%)	11 (19%)	<0.001

Table 11. In the 35-39 age group, pregnancy rates (positive BhCG test), clinical pregnancy rate (with gestational sac documented sonologically) miscarriage rate and live birth rate per cycle and per embryo transfer (where number of cycles in the PGT-A group = 96, number of embryo transfers in the PGT-A group = 77, number of cycles and embryo transfers in the non-PGT-A = 31)

	35-39 age group		p value
	PGT-A	non-PGT-A group	
	n (%)	n (%)	
Pregnancy rate per cycle	45 (46.9%)	9 (29.0%)	0.081
Pregnancy rate per ET	45 (58.4%)	9 (29.0%)	0.006
Clinical pregnancy rate per cycle	34 (35.5%)	8 (25.8)	0.323
Clinical pregnancy rate per ET	34 (44.2%)	8 (25.8)	0.077
Miscarriage rate per cycle	4 (4.20%)	1 (3.20%)	0.304
Miscarriage rate per ET	7 (6.70%)	1 (3.20%)	<0.001
Live birth rate per cycle	30 (31.3%)	7 (22.60%)	0.304
Live birth rate per ET	45 (42.9%)	7 (22.60%)	<0.001

Table 12. In the 40 and above age group, pregnancy rates (positive BhCG test), clinical pregnancy rate (with gestational sac documented sonologically) miscarriage rate and live birth rate per cycle and per embryo transfer (where number of cycles in the PGT-A group = 66, number of embryo transfers in the PGT-A group = 28, number of cycles in the non-PGT-A group = 23 and number of embryo transfers in the non-PGT-A group = 27)

	40 and above age group		p value
	PGT-A	non-PGT-A group	
	n (%)	n (%)	
Pregnancy rate per cycle	20 (30.3%)	5 (21.7%)	0.431
Pregnancy rate per ET	20 (71.4%)	5 (18.5%)	<0.001
Clinical pregnancy rate per cycle	18 (27.2%)	5 (21.7%)	<0.602
Clinical pregnancy rate per ET	18 (64.3%)	5 (18.5%)	<0.00.1
Miscarriage rate per cycle	3 (4.5%)	1 (4.3%)	0.777
Miscarriage rate per ET	3 (10.7%)	1 (3.7%)	<0.001
Live birth rate per cycle	15 (22.7%)	4 (17.4%)	0.777
Live birth rate per ET	15 (53.6%)	4 (14.8%)	<0.001

group, but the result may not be valid due to more than 20% of cells having an expected count of less than 5 (Tables 10, 11, and 12).

One hundred thirty-five women aged 35 and above who underwent 162 single embryo transfer cycles were included in the PGT-A arm. Excluded in this group were those women with a history of recurrent pregnancy loss or recurrent implantation failure, those with known parental chromosomal anomaly, and those with sperm that were surgically retrieved. These are the subgroups of patients where aneuploidy is known to be particularly high, hence their exclusion from the study. The number of women who underwent IVF with day 5 single frozen embryo transfer of morphologically chosen embryos was much fewer (total of 54), and this did

not reach the computed minimum sample size per age tier, particularly for the control group age 40 and above.

Advanced maternal age is a common factor for infertility in all the couples included in this study. Advanced maternal age results in infertility mainly due to a decrease in oocyte competence. Forty-one percent of the women in the present study are age 40 and above. In other countries, where third party IVF is acceptable, some of the women included in the present study would have been offered egg donation instead of undergoing an ovarian hyperstimulation cycle. This option is not allowed in the Philippines, hence limiting the reproductive options of advanced maternal age women, especially those with poor ovarian reserve.

Male factor was a contributory factor present in only 29.6% of couples included, but note that those with severe male factor and those with surgically retrieved sperm were excluded from this study. Anovulation was found to be the most common female co-factor in the advanced maternal age women included in this study.

Women nowadays are focused on education and career advancement during their younger years and consequently delay childbearing.[34] Advanced maternal age results in a decrease in oocyte quality and quantity, with a decrease in oocyte competence leading to infertility, increased risk of miscarriage, and increased incidence of birth anomalies.[35,36] A few of the underlying mechanisms thought to cause a decrease in oocyte competence include shortening of the telomere, cohesins dysfunction, instability of spindle cells, hormonal changes related to age, and dysfunction of the mitochondrial DNA.[36,37] These lead to faulty chromosomal segregation during meiosis resulting in oocyte aneuploidy.[38]

Comparing fertility between women aged 20-24 and 35-39 years old, fertility is reduced by as much as 35% in the latter group. Fertility is known to further rapidly decrease thereafter.[39] It is unfortunate that oocyte aging cannot be halted, reversed, or remedied. The reproductive medicine doctor is then left to propose different therapeutic approaches that may improve fertility outcomes in this population of women who are desirous of pregnancy.[36] According to local database, women with advanced maternal age comprised 64% of all women who underwent IVF at CARMI during the past 10 years. The average age of women who underwent IVF was computed at 36.4 years old. This is comparable with the 2019 data of the Society for Assisted Reproductive Technology showing 63% of women undergoing IVF are considered to be of advanced maternal age.[40]

The control group size in this study was notably small. The control group, which included women aged 35 and above who had single frozen embryo transfer of untested embryos, was deemed the proper control group to eliminate the pregnancy advantage of transferring 2 or more embryos at a time. This small dataset may be partially explained by the self-funding of IVF in this country, where financial burdens and economic pressures may favor the transfer of multiple embryos at a time. The perceived reason for couples' reluctance in doing single embryo transfers includes wanting to enhance the likelihood of pregnancy with reduced cost. The self-pay arrangement of IVF services in this country makes practitioners heavily consider the couples' desires for double or multiple embryos per transfer. This is most especially true in the lower prognosis, advanced maternal age group of patients. The possibility of twins and higher order multiple gestations are explained to them, and most couples deem the risk acceptable. A thorough discussion of ethical and medical issues related to double or multiple embryo transfers enables couples to make a fully informed decision. It must also be noted that the decision of some couples to eventually undergo single embryo transfer of untested embryos was due to lack of choice because only a single embryo was available for transfer. This was true in 18 of 54 patients who did not undergo PGT-A. So although elective single embryo transfer of blastocysts has notable advantages (particularly reduced multiple gestation), a potentially higher pregnancy rate with multiple embryo transfers especially in the older maternal age group pushes the decision to transfer more than 1 embryo at a time. The recommendation of doing elective single embryo transfers may not apply to this patient population. Maximizing the pregnancy chances in one given cycle with minimalization of costs may be the direction of most couples and physicians when they decide to transfer more than 1 blastocyst.[41]

To improve outcomes of single embryo transfers, other selection strategies aside from choosing based solely on morphological grading are proposed.

Determining embryo quality is key, as identifying an embryo with the highest implantation potential decreases the time to pregnancy. Pushing embryos to day 5, in itself, is a form of embryo selection as roughly half of day 3 embryos would not make it to blastocyst stage.[42] Genomics, proteomics, transcriptomics, metabolomics,[28] morphokinetics using time-lapse imaging technology are tools that hold promise in embryo selection.[43] The use of PGT-A for this purpose is one strategy that is also being extensively investigated and is already widely used worldwide. The elective transfer of single embryos found to be euploid by PGT-A is seen by some authors as an effective strategy to implement a single embryo transfer policy. Its use in poor prognosis patients, however, still remains controversial.[45]

In this group of advanced maternal age patients, an expected significant finding is the difference in the number of blastocysts between those aged 35-39 and 40 and above (3.45 ± 2.346 versus 2.61 ± 2.219, p = 0.008). This finding in the present study is an anticipated difference as a result of age, as there is a known decrease in number of blastocysts in those who are older due to aneuploidy.[46] Historically, it was a clinical question whether the earlier technique of doing the biopsy on day 3 affects embryo blastulation.[47] There was evidence of reduction of sustained implantation rates when biopsy was done on day 3 (30% vs 50% for the unbiopsied day 3 embryos). Sustained implantation rates however, were found to be similar in those that underwent day 5 biopsy (51% vs. 54%) versus those that were not biopsied. According to a study by Scott et al[48], biopsy of day 5 embryos is safe, and the procedure has no measurable effect on clinical outcomes.

According to age stratification, there was a higher number of blastocysts per patient in the PGT-A group versus the non-PGT-A group (3.22 ± 2.48 versus 2.74 ± 1.76) although the finding was not statistically different. The final decision on whether PGT-A will be performed is on day 5 after fertilization. This may present a significant favorable selection bias for the PGT-A group, presumably because couples with more blastocysts have room to choose from a bigger lot of embryos to transfer and hence opt for PGT-A. This is in contrast with those who have fewer embryos who may opt to do an outright transfer than spend for PGT-A. Literature shows that overall, blastocyst conversion rate in cycles is 52-83%, with blastulation rate as low as 45% in women over 42 years old.[49] Blastocyst conversion rate and euploid blastocyst development are inversely correlated with maternal age.[50,51] Euploid blastocyst conversion rate was 28% in those younger than 35 years old, to 0% in those older than 42 years old.[52] This may present as a tricky predicament in the advanced maternal age group women who are expected to have a lower yield of oocytes. They may have a lower number of embryos that can endure to blastocyst stage, at which point they can be subjected to a day 5 trophoectoderm biopsy for PGT-A.

In this study, the overall euploid rate was computed at 38.5 percent. The euploidy rate in this study was much lower in the older age group compared to the 35-39 age tier (16.9% versus 47.6% respectively, p value = 0.001). This is parallel to the finding that embryo aneuploidy reaches 80% in women over 40 years old.[53] This compares with the trend of decreased blastocyst euploidy rate as a woman ages, from 60% at 35 years old, 36% at 40 years old, and 24% at 43 years old.[54] According to a review by Vaiarelli et al[55], mathematically prognosticating from the point of egg collection, an estimate of 5 mature eggs for those aged 35-37, 7 mature eggs for those aged 38-40, 10 mature eggs for those aged 41-42 and 20 mature eggs for those greater than 42 years of age are necessary to guarantee at least 1 euploid embryo.

Once a euploid embryo has been selected, a good quality embryo has an 80-90% sustained implantation rate.[56] This is regardless of maternal age.[57,56] The present study shows that euploid embryos transferred do not have a statistically significant difference in pregnancy rate, miscarriage rate and live birth rate (Table 9). This is validated by another study that concluded that if an embryo of a woman aged 35-42 years old is euploid, it has an implantation rate of 40 - 51% and 32 – 44 % for a day 5 and day 3 biopsied embryo, respectively. This is the basis for the guideline of the Preimplantation Genetics Diagnosis International Society (PGDIS) to transfer only 1 euploid at a time regardless of maternal age.[58] In selecting which euploid embryo to transfer, morphodynamic characteristics, that is, blastocyst morphology and speed of development, are useful tools to consider.[59]

How do we explain a non-pregnancy in patients transferred with a good quality euploid embryo? An undetected chromosomal abnormality or mosaicism may be the underlying reason for a non-pregnancy, undetected by the type of platform used for analysis. Next generation sequencing is able to detect mosaicism more frequently than other platforms, but may also screen out embryos that can potentially result in a live birth.[60] Negative beta-hCG results may also be attributed to trauma secondary to the biopsy, the embryo transfer technique, or the endometrial preparation and receptivity.[56] Lower pregnancy rates have been documented from lesser quality euploid embryos, which implies that these embryos may not endure the biopsy procedure as well as those that are of good quality.[61] This last reason is probably why some clinicians transfer 2 low grade euploid embryos despite the recommendation of transferring only 1 euploid embryo at a time, regardless of the age of the woman.[56]

A large proportion of cycles in the present study, 41.4% (67/162), did not yield euploid embryos for transfer. According to age tier, 62.1% occurred in the older age group, and the risk was significantly lower at 27.1% in those aged less than 40. Among the abnormal embryos, 8 were mosaic embryos. As expected, the age of the women at the time of retrieval inversely affects the number of euploid embryos.[57] Hence, the proportion of cycles where no embryos are transferred due to absence of an euploid embryo suitable for transfer, increases with maternal age as well. In a study by Sanders[17], the risk of no transfer increased from 12.5% in the 35–37 age group to a high of 50% in those older than 44 years. For patients above 40 with zygotes less than 4 pronuclei, it is predicted that the risk of having no embryos for transfer was as high as 99%.[50] Patients with advanced maternal age should be advised regarding this risk, as pushing for PGT-A carries the possibility that there will be no embryos available for transfer.

A study done by Herlihy revealed that for those that did not have a euploid to transfer in a previous cycle, doing a second round of stimulation has a 56% chance of obtaining a euploid embryo across all age groups. The euploidy rate of the second cycle was found to be comparable with national age-based statistics and again, tightly related to age (81% in those younger than 35 years old, and 25% in those more than 42 years old). The study concluded that the poor outcome of only aneuploid embryos during a first IVF cycle should not discourage couples from another cycle and that couples should be counseled corresponding to age-appropriate outcomes.

There are proponents that PGT-A has false positive (falsely aneuploid) results stemming from the sampling method. It is possible that the biopsy procedure has a detrimental effect on the embryo, leading to an aneuploid finding. This is particularly true in embryos where the biopsy was done while they were still hatching or when they were not fully expanded.[63] This is relevant in the poor prognosis patients, such as those with advanced maternal age, who have significantly fewer embryos.[64]

It is also claimed that 5-10 cells from the trophoectoderm poorly represent the inner cell mass which is the cellular make-up of the embryo.[65] This claim is supported by reports of healthy live births stemming from embryos that were classified as aneuploid.[66] This assertion is further validated by a study where embryos were biopsied but transferred according to morphology (PGT-A results unknown at the time of transfer) revealing a live birth rate of 8% in the aneuploid group. The pregnancy outcomes were significantly and expectedly poorer in this "aneuploid" group, but healthy newborns were borne from this cohort of embryos nonetheless.[67] These positive pregnancy outcomes may stem from a false positive classification of aneuploidy or from a mechanism known as autocorrection.[31] In another study, whole genome amplification products of embryos previously tested using array comprehensive genomic hybridization were retested using next generation sequencing, and embryos originally tagged as aneuploid were diagnosed mosaic with the newer technique. A proportion miscarried after embryo transfer, but others resulted in live births. In another institution, embryos that underwent testing and were classified as aneuploid by next generation sequencing were transferred to a different center because of the initial center's refusal to transfer aneuploid embryos. The transfer of these "abnormal" embryos resulted in 7 healthy live births and 1 child with coarctation of the aorta. The authors concluded that many couples with only aneuploid embryos are willing to have their aneuploid embryos transferred, as these embryos can still possibly result in healthy live births.[68] So

the question of what to do with aneuploid embryos hangs in the air: should they be retested or should they be disposed of, remains unanswered. More so, we have to ponder over the question of whether it is ethically acceptable to transfer an aneuploid embryo, especially if the patient insists.

Guidelines exist with regard to the transfer of embryos diagnosed as mosaic. Mosaicism is when there are 2 or more cell populations with dissimilar genotypes in 1 embryo.[60] These are embryos with 20-80% aneuploid DNA in a trophoectoderm biopsy and are considered intermediate between a full aneuploid and a euploid embryo.[58] Mosaicism is reported to be detected up to 5-15% of the time and is thought to derive from errors that occur during post-zygotic mitosis, depending on the type of platform used for PGT-A.[69] Mosaic embryos donated for research showed that low-range mosaic embryos have full euploid complement upon biopsy of the inner cell mass, whereas those that showed high-range mosaic embryos were fully aneuploid on further biopsy.[6,70,71] There were also embryos that were classified as euploid by array comprehensive genomic hybridization but miscarried, and on reanalysis of sampled specimen turned out to be mosaic on next generation sequencing. The same researchers also found that in that study, the diagnosis of mosaicism was reproducible in the same embryo only 58.2% of the time.[60] The clinical relevance of mosaicism lies on which chromosome is affected and where the errors are found,[72] especially in patients where only a mosaic embryo may be the only available embryo. It is proposed that mosaicism of the trophoectoderm may not truly reflect the status of the intracellular mass, which forms the fetus.[60] Adequate counseling regarding potential risks and outcomes must be done in cases where there is consideration of transferring mosaic embryos. Studies show that there is no risk of abnormal live births.[73] but with higher implantation failure and miscarriage rates.[67] Mosaicisms found in chromosomes 2, 3, 4 and 15 were those that resulted in live births.[60] If a woman becomes pregnant after a mosaic transfer, none invasive prenatal testing (NIPT) using amniocentesis on or beyond the 14th week is considered the better prenatal test because the sample is considered most aligned with the chromosomal make-up of the fetus.[69]

This study suggests that in the advanced maternal age group, there is an improvement in pregnancy rate, clinical pregnancy rate, and live birth rate per embryo transfer with the use of PGT-A, but not in a per cycle basis. Although there is a trend leaning towards improved rates when applying PGT-A on a per cycle basis, the results are not statistically different from the control group. The authors believe that more patients need to be included to get more robust conclusions.

In good prognosis patients, meta-analyses showed improved pregnancy rates in those that underwent PGT-A leading to increased implantation rates per transfer, clinical pregnancy rates, and live birth rates per cycle in good prognosis patients.[33,45] These findings were opposed by other more recent randomized controlled trials that showed the absence of benefit in terms of pregnancy outcome in the younger patients.[19,28] Ozgur et al[28] attributed the failure to produce positive pregnancy outcomes in good prognosis patients to the inherent limitations of trophoectoderm biopsy. A very recent meta-analysis of 8 studies on the use of PGT-A for women less than or equal to 37 years old showed that the technology does not seem to improve ongoing pregnancy rates and live birth rates as compared to standard morphological embryo assessments.[74]

In the advanced maternal age group of women, an early meta-analysis of PGT-A of cleavage stage embryos analyzed by FISH showed no benefit with this technology.[20] Improvement in techniques including biopsy of day 5 trophoectoderm versus cleavage stage embryos, and an increased number of cells biopsied (comprehensive chromosomal screening) were subsequently employed in the conduct of PGT-A. Subsequently, a study revealed that in women less than 42 years of age, there was a 60.7% ongoing pregnancy rate when 1 euploid blastocyst was transferred, which was equivalent to the pregnancy outcomes of transferring 2 untested blastocysts in a fresh cycle.[75] Present results are consistent with studies showing benefits of the use of PGT-A in the advanced maternal age population, albeit the population size is small. In women over the age of 37, the odds of a clinical gestation per embryo transfer were found to be 3.86 times higher and the odds of a live birth per embryo transfer were 8.2 times higher in those that underwent PGT-A, but no advantage was seen in a per cycle basis.[76] A review of US data showed an improved live birth rate per transfer (greater than 37 years old) and

lower miscarriage rates (35 years old and older)[33] but a re-analysis attributed the improved pregnancy outcome to a selection bias for better prognosis patients in the PGT-A group since more cycles in the PGT-A group reached embryo transfer.[77] A randomized controlled trial showed a higher delivery rate (52.9% versus 24.2%), lower miscarriage rate and a decreased time to pregnancy in the 38-41 year-old population of day 3 embryos, analyzed by array comparative genomic hybridization.[23] Similar to the present study, a retrospective cohort analysis of UK data concluded that PGT-A resulted in higher live birth rates per embryo transfer, and per cycle as well, across all age groups, but more so in the older age group.[17]

The Single Embryo TrAnsfeR of Euploid Embryo (STAR) trial, a large scale, multinational double-blinded randomized controlled trial of 661 women employing PGT-A using next generation sequencing versus a non-PGT-A group showed a significant increase in ongoing pregnancy rate per embryo transfer (51% versus 37%) in women 35-40 years old that used PGT-A. The increase however was not considered significant on a per intention to treat basis.[19] These findings were however refuted due to the exclusion of a significant population of patients, and they claimed that, upon recalculation, there was no improvement in outcomes in this large study even on a per transfer basis.[78] It was also questioned that in analyzing the outcomes of PGT-A on a per transfer basis, the journey to getting to the point of embryo transfer is overlooked.[79]

In the very advanced age group, Ubaldi et a1[1] in a study of 137 women aged 44-47 years old using PGT-A with polymerase chain reaction showed only a 14% euploid blastocyst rate, with a 57.1% delivery rate per embryo transfer, but only an 8.0% delivery rate per cycle. The authors then concluded that this extremely older population has a very low likelihood of pregnancy success because of the high-risk of aneuploidies, but if with good ovarian reserve, must be encouraged to use PGT-A because of the good delivery rates and low miscarriage rates seen when a euploid embryo was available for transfer.

PGT-A is not without risks. There are multiple steps and processes involved in the application of this technology. Sampling errors may occur that are related to the laboratory conditions and skill of the embryologist.[25] Trophectoderm biopsy can bring about iatrogenic damage to the embryo and negatively impact the embryo's potential to implant.[38] It was mathematically computed that there is 30% possible implantation loss when PGT-A is done.[80] This risk of loss may be acceptable if the advanced maternal age woman has many available blastocysts. In that situation, information that can be gained from PGT-A may offset the potential loss of possible implantations in these cases.[76]

Another issue is that the practice of extended in vitro culture may prolong exposure of the embryos to unnatural conditions. In theory, this can cause imprinting and epigenetic changes that may affect embryo development.[81] Long-term developmental and safety outcomes of births that employed PGT-A are still to be reported. Additionally, the ability of the embryo to autocorrect is an issue that has to be addressed and further investigated.[25] The occurrence of mosaic embryos and its transfer is another science on its own. The issues surrounding the insistence of a couple to transfer an aneuploid embryo also pose an ethical and possible legal dilemma.

In countries where IVF is subsidized or covered by insurance, PGT-A is usually an out-of-pocket cost, carrying with it its own selection bias.[79] Locally, it is an added cost that must be personally funded, on top of the costs of an already expensive IVF procedure. A cost-effectiveness study performed in Europe and the United States claimed that the estimated cost-per-live birth may be lower with PGT-A given that it may reduce the number of failed transfers and decrease the time to pregnancy. But costs differ widely among centers and from one country to another. A local cost analysis study should be done before quoting the cost-benefit of PGT-A during counseling and for decision-making. Looking at the big picture, the cost of PGT-A may be an investment worth making since the cost is offset by the increased pregnancy rate and live birth rate per embryo transfer as seen in this study, despite the risk of having no embryos in the cycle. Aside from cost, though, there are other determinants that influence acceptability of PGT-A by couples. These include personal values, religion, social and family support, influences of the clinician, and the patient's previous reproductive history.[82]

One must keep in mind the huge emotional, physical, and financial investment a couple makes

when they undergo IVF. The process can be emotionally and financially draining. Advanced maternal age patients, with established known high percentage of aneuploid embryos, may end up being subjected to multiple unsuccessful embryo transfer, most of which will end in distressing miscarriages and pregnancy losses. The emotional burden that may possibly be lifted while overcoming the negative pregnancy outcomes by performing PGT-A may be worth the cost of this add-on.

Limitations

This study was limited by its retrospective study design. The ideal study design would be a prospective randomized study. The authors were not able to establish the intent to pursue PGT-A before the start of the cycle, hence it is not a true intention-to-treat study. Attrition of patients intending to do PGT-A occurred in many levels.

The small sample size was a limiting factor. Choosing an appropriate control group for this type of study was difficult. A very important limitation was the low number of control subjects, that is, women aged 35 years old and above undergoing IVF with a single morphologically chosen blastocyst in a frozen embryo transfer, hence comparisons between the PGT-A group and the control group may not be valid. The occurrence of miscarriage as a pregnancy outcome was also too few to be significantly compared, and this is due to the small sample size. Those that underwent single embryo transfer were those with only 1 embryo and those that did not want a multigestational pregnancy. There were also those that planned to do PGT-A but because of a disappointingly low embryo yield or poor-quality embryos, opted to transfer an untested day 5 embryo in a frozen embryo transfer instead. This may have presented as a selection bias in favor of the PGT-A group, with more favorable patients eventually opting to do PGT-A. The higher number of blastocysts in the PGT-A group, although not found to be statistically significant, supported this selection bias and may partially explain the observed improved pregnancy rate, clinical pregnancy rate and live birth rate per embryo transfer in the older PGS group.

Conclusion

The debate continues whether there is benefit of PGT-A in the advanced maternal age patients undergoing IVF. The scientific community remains divided into supporters and sceptics of this technology. There is lack of high-quality evidence and this is the reason why this technology remains divisive. Despite it being controversial, it is popular and widely used. PGT-A is considered an add-on that presently has no established or strong benefit in the advanced maternal age group of patients. Standard implementation, as is done in some centers, should be revisited because the data is not robust. Overall, results of studies are dichotomous in terms of outcomes of PGT-A in the advanced maternal age group of women. There are reports of improved clinical birth rates, live birth rates, and ongoing pregnancy rates on a per embryo transfer basis. However, there are meta-analysis that show no improvements of these outcomes.

This study of CARMI patients shows improved pregnancy outcomes in the small population of advanced maternal age patients undergoing IVF with PGT-A. Pregnancy rate and live birth rate per embryo transfer were higher in the PGT-A versus non-PGT-A group (61.9% vs 24.1%0 and 42.9% vs 19%). It suggests that there is an improvement in the pregnancy rate and live birth rate on a per embryo transfer basis. The miscarriage rate in the PGT-A group was higher, but the total number of events was few.

Overall euploid blastocyst rate was 38.5%. It was 16.9% for 40 and above and 47.6% for those age 35-39. This group of patients, because of their high risk of having aneuploid embryos detected by PGT-A, were also at an increased risk for no embryo transfers. Forty-one percent did not have a transfer because of aneuploidy.

One should take into account the possible flaws of the technology in misdiagnosing embryos and the autocorrection of aneuploid embryos. The results presented in this study may help set realistic expectations. Proper and extensive counselling must be provided before a couple employs this technology. Cost, risks, and positive and negative outcomes must be discussed before recommending and employing this technology. PGT-A is an expensive

and invasive technology, and in the local setting, the cost-effectiveness remains uncertain.

Recommendation

Further research, particularly well-designed, randomized controlled trials with large sample sizes are needed to produce high-quality conclusive evidence regarding the use of this widely accepted diagnostic tool. The information garnered from this research may serve as preliminary data that can be used to counsel the patients in the center. There is still a need, however, to definitively establish its clinical benefit in IVF for the advanced maternal age population. Although the use of PGT-A in older women does appear to improve pregnancy rates and live birth rates, patients must be aware that this benefit may not persist when assessed per initiated cycle. A longer period of time to include more subjects and to assess outcomes of multiple embryo transfers within a cycle would also be ideal to gauge cumulative live birth rate. A cost-effectivity study will be beneficial and can give significant information that can be applied for counseling and properly guide the decision-making process of the patients.

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