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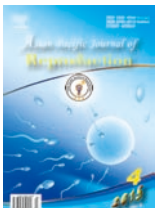

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Aneuploidy does not explain the difference in outcomes observed between Asian and Caucasian patients undergoing *in vitro* fertilization

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ABSTRACT

Objective: To understand whether reduced pregnancy and live birth rates for Asian patients undergoing *in vitro* fertilization (IVF) could be explained by discrepant rates of aneuploid embryos. **Methods:** A retrospective cohort study of all autologous and donor IVF cycles utilizing pre-implantation genetic screening (PGS) at a single infertility clinic from January 2012 to December 2013. **Results:** After controlling for maternal age, there was no difference in aneuploidy rates of Caucasian patients compared to Asian patients'. A trend was discerned that embryos of Caucasians form blastocysts more frequently than those of Asian patients, reaching significance for patients aged 25 to 30 and 40 to 45, but there was no difference in the likelihood of having at least one euploid blastocyst to transfer in any age group. **Conclusion:** While there may be a slight difference in blastocyst formation rates, there is no difference in aneuploidy or euploid blastocyst transfer rates between Asian and Caucasian patients that would explain the discrepancy in IVF outcomes observed between these patient populations. Possible ethnicity specific differences in non-ploidy related embryo viability and endometrial receptivity should be investigated as potential etiologies for this observation.

1. Introduction

As in many health science fields, the development of larger, more granular databases in assisted reproduction has led to the realization of ethnic-specific variations in both diagnostic[1] and therapeutic results[2], forcing clinicians and researchers to re-evaluate their expectation of normal or average based on patient ethnicity. Previous observational studies using a national database have shown that infertile Asian women undergoing *in vitro* fertilization (IVF) have lower pregnancy and live birth rates compared to Caucasian women of similar age, ovarian reserve, and infertility diagnoses, despite similar treatment protocols and apparent treatment responses prior to embryo transfer[3,4]. While this study did not control for day or stage of embryo transfer, these results are consistent with a more recent study that found the odds of Asian patients having a live birth after undergoing IVF with a blastocyst transfer were almost half those of similar Caucasian patients[5].

IVF failure is often blamed on some combination of the inability to produce sufficient quality embryos or the lack of a receptive intrauterine environment. With the advent of better arrays and more commonly performed pre-implantation genetic screening and products of conception analysis, it has become apparent that the majority of assisted reproduction[6] and early pregnancy failures[7], particularly those associated with increasing maternal age, can be explained by embryo aneuploidy. While the Langen *et al*. study[5] limited their analysis to blastocyst transfers in an attempt to control for embryo quality, a significant portion of blastocysts with even the best day 5 morphology are known to be aneuploid[8] and incapable of becoming a genetically normal pregnancy. No prior studies have investigated if there is a difference in aneuploidy rates between Asian and Caucasian patients, and if present, it would explain the observed difference in IVF outcomes and have implications in prenatal screening and other areas of perinatal health. This study aims to investigate if the observed differences in Caucasian and Asian IVF outcomes are attributable to different rates of embryo aneuploidy or blastocyst formation.

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2. Materials and methods

This was a retrospective review of all autologous and donor IVF cycles of patients aged 18–45 using pre-implantation genetic screening with 24-chromosome analysis at a single infertility clinic from January 2012 to December 2013. Female patient ethnicity was self-identified as Asian, Caucasian, Latina, South Asian, or other, but only cycles from patients that were identified as being Asian or Caucasian were included for analysis. In addition to patient ethnicity, cycles were reviewed for maternal or donor age, number of total and mature oocytes retrieved, number of fertilized oocytes, number of blastocysts formed, and PGS results.

With regard to infertility diagnoses, all patients were included and PGS was performed for the indications of advanced maternal age, recurrent implantation failure, family balancing, history of maternal or paternal illness, patient request, or in conjunction with single gene testing. All patients underwent oral contraceptive down regulation followed by ovarian stimulation with antagonist or short agonist protocols. Concurrent ultrasound monitoring was performed every other day as indicated until the lead follicle reached 18 – 20 mm in mean diameter, when either a Lupron and hCG or hCG alone trigger was administered. Oocyte retrieval was performed under transvaginal ultrasound guidance thirty-six hours following hCG or hCG/Lupron administration. A mature, or MII oocyte, was defined by the presence of the first polar body. All oocytes were fertilized via intracytoplasmic sperm injection (ICSI). Embryos were group cultured in SAGE Global medium with 10% SAGE Quinn's Advantage Serum Protein Substitute (SPS) supplementation under low oxygen conditions days 0 – 5. On day 3 following fertilization, all embryos underwent laser assisted hatching, and all non-arrested embryos that were at least a morula by day 5 underwent laser assisted trophectoderm biopsy for PGS on day 5 or 6. After PGS biopsy embryos were individually cultured in SAGE Quinn's Advantage Blastocyst medium with 20% SAGE SPS supplementation. Blastocyst formation was defined by the presence of cavitation.PGS was performed by two commercial PGS providers (Blastogen, Reprogenetics) using array-CGH or SNP array. Data was collected on an excel spreadsheet and analyzed using Chi Square, ANOVA, or Fisher Exact test for analysis.

3. Results

During 2012-2013 there were 381 IVF cycles utilizing PGS from 256 patients. Seventy-eight cycles were excluded as the patients or donors were identified as a non-Asian or non-Caucasian ethnicity. Of the included 303 cycles, 110 included patients or oocyte donors who identified themselves as Asian, and the remaining 193 were from patients or oocyte donors who identified themselves as Caucasian. In total, 2 423 embryos, 734 from Asian patients and 1 689 from Caucasian patients, were included for analysis.

Considering the entire population, the cohort of Caucasian patients had a greater proportion of oocyte donors and was therefore significantly younger than the Asian cohort (Table 1) and they also had more blastocysts per patient. After stratifying patients based on age, there was no significant difference in mature oocyte yield (Table 2) or fertilization rate of mature oocytes (Table 3) between Asian and Caucasian patients, but there was a trend that the embryos of Caucasian patients were more likely to reach the blastocyst stage (Table 4), although this trend only reached statistical significance among patients ages 25 to 30 and 40 to 45.

There was no trend or statistically significant difference in the aneuploidy rates of embryos between Asian and Caucasian patients (Table 5). There was a trend among the youngest patient population of Asian patients having a slightly lower probability of being able to proceed with a euploid blastocyst transfer, although this trend was not consistent in patients over 35 years old, and did not reach statistical significance in any patient population (Table 6).

Table 1
Patient characteristics.

Parameters	Asian (n=110)	Caucasian (n=193)	All (n=303)	P value
Age (Yrs)	33.9 ± 7.8	28.4± 6.3	30.4 ± 7.4	<0.05
# Donor cycles (%)	44 (40%)	147 (76%)	63%	<0.05
# Blastocysts (avg. per pt)	734 (6.7)	1689 (8.8)	2423	<0.05

Table 2
Oocytes retrieved.

Age	Mature oocytes retrieved			P value
	Asian	Caucasian	All	
<25	15.8	18.5	17.8	0.13
25 to 30	11.7	15.3	14.6	0.10
31 to 35	13.8	14.4	14.2	0.82
36 to 40	9.6	10.1	9.8	0.79
40 to 45	7.9	6.6	7.4	0.25

Table 3
Fertilization rate.

Age	Fertilization rate (2pn per MII)			P value
	Asian	Caucasian	All	
<25	71%	69%	69%	0.17
25 to 30	99%	80%	84%	0.20
31 to 35	70%	73%	72%	0.64
36 to 40	85%	67%	78%	0.09
40 to 45	84%	71%	79%	0.26

Table 4
Blastocyst formation rate.

Age	Blastocyst formation rate			*P* value
	Asian	Caucasian	All	
<25	78%	83%	82%	0.09
25 to 30	70%	82%	80%	<0.05
31 to 35	79%	77%	78%	0.58
36 to 40	89%	92%	90%	0.56
40 to 45	83%	94%	86%	<0.05

Table 5
Euploid rate.

Age	Euploid rate			*P* value
	Asian	Caucasian	All	
<25	66%	49%	49%	0.10
25 to 30	48%	50%	50%	0.71
31 to 35	40%	39%	40%	0.84
36 to 40	23%	16%	20%	0.21
40 to 45	15%	7%	12%	0.07

Table 6
Euploid blastocyst transfer rate.

Age	Euploid blastocyst transfer rate			*P* value
	Asian	Caucasian	All	
<25	92%	99%	97%	0.08
25 to 30	92%	98%	97%	0.24
31 to 35	89%	91%	90%	0.89
36 to 40	76%	64%	72%	0.45
40 to 45	38%	28%	35%	0.45

4. Conclusions

Observational data has suggested that Asian ethnicity is a risk factor for poor IVF outcomes, although no clear etiology of this discrepancy has been demonstrated. Aneuploidy is often blamed for IVF treatment failures in cycles without PGS, and in the current study we investigated whether discrepant aneuploidy rates among Asian patients compared to Caucasian patients might explain the difference in IVF outcomes. In this cohort of patients, there was no difference in blastocyst aneuploidy rates between Asian and Caucasian women. While embryos from Caucasian patients were more likely to reach the blastocyst stage in some age groups, the likelihood of an Asian patient being able to proceed with a euploid blastocyst transfer, which is more clinically relevant to pregnancy and live birth rates, was similar to age matched Caucasian counterparts at every age. This suggests that non-ploidy determinants of embryo viability and endometrial receptivity to a transferred euploid blastocyst may potentially explain the poorer pregnancy and live birth rates observed in Asian patients undergoing IVF.

In addition to the limitation of size and its retrospective design, this study is also limited by the fact that ploidy information was only available on embryos reaching the blastocyst stage and available for trophectoderm biopsy. In view of the fact that there was a trend for Caucasian patients' embryos reaching blastocyst more frequently than the embryos of Asian patients, it is plausible that most of the embryos not eligible for PGS were aneuploid, and their absence from our ploidy data may have biased our results towards a lower aneuploidy rate for Asian patients. However, the similar probabilities of Asians and Caucasians being able to proceed with a euploid embryo transfer, suggests that non-ploidy related embryo characteristics or endometrial receptivity could be more likely explanations of the discrepant IVF outcomes between Asian and Caucasian patients. Additionally, patient ethnicity was self-reported and therefore subject to reporting bias. Moreover, the inability to capture patients with some degree of mixed ethnicity could have also skewed the data. Patients also were only stratified by maternal age, as this is the most consistently demonstrated determinant of aneuploidy rates; however, there may be other confounding factors that could affect aneuploidy, that were not controlled for in our analysis.

Data regarding endometrial thickness, serum estradiol, and progesterone were not available for these patients, but prior studies demonstrating poorer IVF outcomes among Asian patients have demonstrated that Asian women had significantly higher serum estradiol levels on the day of hCG trigger, a thicker endometrium, and even a subjectively more difficult embryo transfer than Caucasian patients[3], suggesting that elements of IVF other than the embryo may contribute to disparate outcomes for Asian women. It will be interesting to stratify patients by ethnicity as studies of new assays of endometrial receptivity are increasingly validated[9]. Furthermore, other factors that may impact embryo implantation, such as endometriosis or patient BMI, were not captured and while not consistently described, some studies have demonstrated an increased prevalence of endometriosis[10] and manifestations of adverse effects of obesity at a lower range of 'normal' BMI values for Asian women[11].

This preliminary study on ethnicity specific aneuploidy rates is reassuring that after controlling for maternal age, embryo aneuploidy does not differ between Asian and Caucasian patients, however larger studies, possibly via patient registries, are needed for confirmation as the implications go beyond assisted reproduction. Should any differences be demonstrated, perinatal diagnostics and state screening programs would need to adjust algorithms to reflect ethnicity-specific risks for aneuploidy. Additional study of ethnicity specific aneuploidy rates, endometrial receptivity, and other aspects of assisted reproduction are needed to better understand the observed ethnicity specific variations in treatment outcomes, so that

any disparities can be recognized and ultimately overcome with treatment modifications.

Conflict of interest statement

We declare that we have no conflict of interest.

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