Blastocyst euploidy and implantation rates in a young (<35 years) and old (≥35 years) presumed fertile and infertile patient population

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Objective: To examine the relationship between blastocyst euploidy and implantation rates in a presumed fertile patient population.
Design: Retrospective analysis.
Setting: Private IVF clinic.
Patient(s): IVF patients undergoing comprehensive chromosome screening (CCS).
Intervention(s): Embryo biopsy at the blastocyst stage with preimplantation genetic screening using CCS.
Main Outcome Measure(s): Euploidy, chemical pregnancy, and implantation rates.
Result(s): There was no significant difference in the number of euploid blastocysts between presumed fertile (68/118, 57.6%) and infertile (75/132, 56.8%) patients <35 years old. Likewise, there was no significant difference in the number of euploid blastocysts between presumed fertile (42/86, 48.8%) and infertile (97/206, 47.1%) patients ≥35 years old. When those same patients underwent a corresponding frozen embryo transfer cycle, presumed fertile patients demonstrated a significantly higher chemical pregnancy rate when compared with infertile patients, 28/33 (84.8%) and 50/81 (61.7%), respectively. Moreover, presumed fertile patients exhibited significantly higher implantation rates compared with infertile patients, 36/42 (85.7%) and 54/109 (66.7%), respectively.
Conclusion(s): When subdivided by maternal age, no significant difference was seen in blastocyst euploidy rates between presumed fertile and infertile patients; however, chemical pregnancy and implantation rates were significantly higher in a presumed fertile patient population even when transferring only euploid blastocysts. This would indicate that infertility, as a disease, may encompass other aspects such as uterine or other unknown embryological factors that can influence outcomes. (Fertil Steril® 2014;102:1318–23. ©2014 by American Society for Reproductive Medicine.)
Key Words: Preimplantation genetic screening, aneuploidy, embryo biopsy, comprehensive chromosome screening, IVF

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One of the factors contributing to the lack of success in IVF is the high incidence of whole chromosomal abnormalities or aneuploidies in the developing embryo (1). Preimplantation genetic screening (PGS) was adopted to test for aneuploidies in preimplantation embryos to decrease miscarriage rates and increase live-birth rates. This concept was first applied to specific patient groups who were believed to be more prone to aneuploidies, such as patients diagnosed with recurrent pregnancy loss, multiple IVF failures, previous aneuploid conceptions, male factor, or patients of advanced maternal age (2–7). Studies have indicated that a majority of embryos, regardless of patient diagnosis, contain aneuploidies (8–11). Because of the high incidence of aneuploidies during human preimplantation development, screening the embryos before transfer makes sense, as a majority of miscarriages and embryo wastage are derived from chromosomal aneuploidies (12).

Since the majority of embryos analyzed during the course of IVF are derived from infertile couples or from discarded embryos, the true nature of

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preimplantation aneuploidy may be overrepresented (13). The only embryos that may reflect the true incidence of preimplantation aneuploidy would be those derived from fertile, not infertile, patients. Unfortunately, IVF is not heavily used by fertile patients; however, there are specific patient groups that are presumed to be fertile. For example, anonymous oocyte donors offer insights into aneuploidy rates in young and presumed fertile patients but fail to yield aneuploidy rates in an older (≥35 years) presumed fertile patient population. Therefore, patients who are ≥35 years old and undergoing IVF for single-gene disorder in combination with aneuploidy screening or social sex selection offer a glimpse into the preimplantation aneuploidy in an older, presumed fertile population. Given that the incidence of aneuploidy is patient dependent, it is possible that fertile patients may not exhibit the same rate of aneuploidy that is seen in infertile women of the same age (14, 15).

The purpose of this study is to test the hypothesis that infertile patients exhibit a higher rate of aneuploidy and lower implantation potential of euploid blastocysts compared with a presumed fertile population. This study compares blastocyst euploid rates in presumed fertile and infertile patients, both from a young (<35 years) and old (≥35 years) female patient population. Furthermore, this study will compare pregnancy and implantation rates of the same presumed fertile and infertile patients in a corresponding frozen embryo transfer (FET) cycle.

MATERIALS AND METHODS

This study was deemed exempt by the Sterling Institutional Review Board (Atlanta, Georgia). Only patients attending Reproductive Endocrinology Associates of Charlotte for IVF and comprehensive chromosome screening (CCS) at the blastocyst stage, using bacterial artificial chromosome array comparative hybridization, single-nucleotide polymorphism microarray, or quantitative polymerase chain reaction between January 2010 and January 2014 were included in this study. Patients were divided into two categories, presumed fertile and infertile. Presumed fertile patients were those undergoing IVF either with anonymous oocyte donor, social sex selection, or single-gene defect (excluding fragile X syndrome). Infertile patients included all other diagnoses. To gain further insight into the effect of age on aneuploidy, patients were subdivided based on maternal age, <35 and ≥35 years old.

Kruskal-Wallis, Mann-Whitney *U*-test, χ^2, and Fisher's exact test were used, and $P<.05$ was considered statistically significant.

Embryo Culture

Oocytes were retrieved, stripped of cumulus cells, separated by maturity, and placed into individual 250-μL drops of continuous single culture (CSC; Irvine Scientific) media supplemented with 10% serum substitute supplement (SSS; Irvine Scientific) overlaid with oil (Irvine Scientific). The dish with the oocytes was placed into an incubator at 37°C with 5% CO_2, 95% N_2, and 95% humidity for 2–3 hours until intracytoplasmic sperm injection (ICSI).

ICSI was performed on all mature oocytes as described by Nagy and colleagues (16). Those with two pronuclei at 16–18 hours after ICSI were separated into a separate dish of CSC + 10% SSS, overlaid with oil and placed back into the incubator. On day 3, all embryos underwent assisted hatching using a laser (Zilos-tk, Hamilton Thorne). Two to three laser shots at a pulse of 610 μs were used to breach the zona pellucida. Embryos were placed back into the incubator and allowed to culture until day 5 or day 6.

Those blastocysts that were observed to have a good- or fair-quality inner-cell mass and trophectoderm that was protruding from the zona pellucida were biopsied and vitrified the same day. When desired by the patients, those embryos with a poor-quality inner-cell mass or trophectoderm were biopsied and vitrified. Embryos were given until day 6 to reach the blastocyst stage; those that did not blastulate by day 6 were discarded.

Trophectoderm Biopsy

Blastocysts were placed in a drop of modified human tubal fluid (Irvine Scientific) + 10% SSS. The protruding trophectoderm was aspirated into the biopsy pipette (Humagen). Laser pulses of 610 μs were used to "cut" the trophectoderm, taking care to minimize the number of laser shots necessary. Reference labs were used for all CCS procedures; therefore the piece of trophectoderm was prepared according to their protocols.

Vitrification

Immediately after biopsy, blastocysts were individually vitrified. Blastocysts were placed in equilibration solution (Irvine Scientific) for 15 minutes and then transferred to vitrification solution (Irvine Scientific) for <1 minute. Blastocysts were pipetted onto a Cryolock (Biodiseno), plunged into liquid nitrogen, and capped.

Warming and Transfer

Only blastocysts with euploid results were warmed in a subsequent FET cycle. The Cryolock containing the blastocyst was uncapped under liquid nitrogen and plunged into 37°C thawing solution (Irvine Scientific) for 1 minute. The blastocyst was transferred to dilute solution (Irvine Scientific) for 3 minutes and finally washing solution (Irvine Scientific) for 10 minutes before being placed into CSC + 20% SSS overlaid with oil.

For FET cycles, the endometrial lining was prepped with estrogen patches for approximately 12 days. Progesterone in oil was administered (day 0) when the endometrial lining was ≥8 mm. The blastocysts were warmed on the sixth day of progesterone administration. After warming, blastocysts were transferred to the uterus with a Wallace catheter (Smiths Medical) under ultrasound guidance. Patients continued daily progesterone in oil shots until a negative pregnancy test or 8 weeks of gestation.

RESULTS

Only patients who underwent an FET cycle of a euploid blastocyst were included in the aneuploidy analysis.

Aneuploidy in Presumed Fertile and Infertile Patients <35 Years Old

A total of 50 CCS cycles from 42 patients were performed on women <35 years old. Eighteen (31.5 ± 2.2 years; range, 21–34 years) presumed fertile patients underwent 21 IVF cycles and 24 infertile (32.1 ± 2.1 years; range, 25–34 years) patients underwent 29 IVF cycles. Twenty-one presumed fertile patients underwent CCS for the following reasons: single gene (n = 6), gender selection (n = 8), and anonymous oocyte donor (n = 7). Of those embryos derived from patients with single-gene disorders, embryos may be diagnosed euploid but may be affected with the single gene in question; therefore, those embryos were not available for transfer. The primary diagnoses for the 29 infertile cycles were as follows: polycystic ovarian syndrome (PCOS; n = 4), severe male factor (n = 6), recurrent pregnancy loss (RPL; n = 9), diminished ovarian reserve (n = 1), unexplained (n = 5), secondary infertility (n = 2), primary infertility (n = 1), and endometriosis (n = 1). There was no significant difference between E_2 level at hCG, day of hCG, or total IUs of gonadotropins used between presumed fertile and infertile patients <35 years old (Table 1). There was no significant difference among maternal age, average number of oocytes, average number of embryos, and number of euploid blastocysts between presumed fertile and infertile patients <35 years old (Table 1). However, a significantly higher percentage of blastocysts were biopsied from the presumed fertile group (118/244, 48.4%) than from the infertile group (132/340, 38.8%; $P<.0001$; Table 1).

Aneuploidy in Presumed Fertile and Infertile Patients ≥35 Years Old

A total of 66 CCS cycles were performed on embryos derived from women ≥35 years old. Seventeen cycles from 13 presumed fertile (37.6 ± 1.9 years; range, 35–42 years) patients and 49 cycles from 38 infertile (38.3 ± 2.2 years; range, 35–44 years) patients underwent IVF with blastocyst biopsy. Seventeen presumed fertile cycles underwent CCS for the following reasons: gender selection (n = 12) and single gene (n = 5). The primary diagnoses for 49 infertile cycles were unexplained (n = 13), diminished ovarian reserve (n = 8), endometriosis (n = 2), severe male factor (n = 4), PCOS (n = 4), RPL (n = 8), secondary infertility (n = 2), uterine factor (n = 2), and advanced maternal age (n = 6). E_2 levels on the day of hCG administration were not significantly higher in the presumed fertile group when compared with the infertile group (Table 2). Total gonadotropins used during the stimulation cycle were significantly lower in the presumed fertile group when compared with the infertile group, 2,660.4 ± 1,039.8 IU and 3,749.3 ± 1,362.6 IU, respectively ($P=.0061$). There was no difference in the number of stimulation days, number of oocytes produced, number of embryos generated, and number of blastocysts biopsied between presumed fertile and infertile patients (Table 2). There was no difference in the number of euploid blastocysts between presumed fertile patients (42/86, 48.8%) and infertile patients (97/206, 47.1%; $P=.8852$; Table 2). The average number of euploid blastocysts was also not significantly different between the presumed fertile group and the infertile group (Table 2).

Implantation

Presumed fertile and infertile patients were grouped together when comparing pregnancy and implantation rates in a corresponding FET cycle. A total of 114 FETs of euploid blastocysts were analyzed. Thirty-one presumed fertile patients underwent 33 FETs, while 62 infertile patients underwent 81 FETs. The primary diagnoses of all the cycles undergoing an FET are presented in Table 3. Maternal age at time of retrieval was not statistically significant between presumed fertile and infertile patients, 34.2 ± 3.8 years and 35.5 ± 3.6 years, respectively ($P=.1168$; Table 4). All patients were urged to transfer only one euploid blastocyst; however, some patients decided to transfer two euploid blastocysts based on their medical history. Regardless, the average number of euploid blastocysts transferred in an FET cycle was not

TABLE 1

Cycle characteristics between presumed fertile and infertile patients <35 years old.

	Presumed fertile	Infertile	*P* value
No. of patients	18	24	
No. of cycles	21	29	
Maternal age at retrieval ± SD	31.5 ± 2.2	32.1 ± 2.1	.3470[a]
E_2 at hCG ± SD	3,865.5 ± 1,413.6	4,526.8 ± 1,708.6	.2306[a]
Total IU of gonadotropins ± SD	2,787.3 ± 1,188.4	2,782.2 ± 1,261.3	.8983[a]
Day of hCG ± SD	9.6 ± 1.2	9.8 ± 1.1	.4818[a]
Average no. of eggs	17.2 ± 6.8	18.1 ± 7.3	.7377[a]
Average no. of embryos	11.6 ± 5.6	11.7 ± 5.4	.9057[a]
No. of eggs	361	525	—
No. of embryos	244	340	—
No. of blasts biopsy (%)	118 (48.4)	132 (38.8%)	<.0001[b]
Total euploid (%)	68 (57.6%)	75 (56.8)	.9992[b]
Average no. of euploid (%)	3.2 ± 2.0	2.4 ± 1.9	.1702[a]

[a] Mann-Whitney *U*-test.
[b] χ^2-test for independence.

Taylor. Fertile and infertile implantation rates. Fertil Steril 2014.

TABLE 2

Cycle characteristics between presumed fertile and infertile patients ≥35 years old.

	Presumed fertile	Infertile	*P* value
No. of patients	13	38	
No. of cycles	17	49	
Maternal age at retrieval ± SD	37.6 ± 1.9	38.3 ± 2.2	.4643[a]
E_2 at hCG ± SD	4,455.1 ± 2,252.9	3,852.0 ± 2,081.5	.2979[a]
Total IU of gonadotropins ± SD	2,660.4 ± 1,039.8	3,749.3 ± 1,362.6	.0061[a]
Day of hCG ± SD	9.7 ± 1.4	10.5 ± 1.3	.0620[a]
Average no. of eggs	17.3 ± 7.3	16.7 ± 6.9	.8086[a]
Average no. of embryos	11.5 ± 4.7	9.8 ± 4.7	.3140[a]
No. of eggs	294	819	—
No. of embryos	195	482	—
No. of blasts biopsy (%)	86 (44.1)	206 (42.7)	.8112[b]
Total euploid (%)	42 (48.8)	97 (47.1)	.8852[b]
Average no. of euploid (%)	2.5 ± 1.4	2.0 ± 1.4	.3478[a]

[a] Mann-Whitney *U*-test.
[b] χ^2-test for independence.

Taylor. Fertile and infertile implantation rates. Fertil Steril 2014.

significantly different between presumed fertile (1.3 ± 0.5 blastocysts) and infertile (1.4 ± 0.5 blastocysts) patients (Table 4; *P*=.5695). There was also no significant difference in embryo quality transferred between the two groups. Chemical pregnancy rates, as defined by a positive beta hCG level, were significantly higher in the presumed fertile patients when compared with the infertile patients, 28/33 (84.8%) and 50/81 (61.7%), respectively (Table 4; *P*=.0251). Clinical pregnancies, as defined by the presence of a gestational sac, were significantly higher in the presumed fertile (28/33, 84.8%) patients compared with the infertile patients (44/81, 54.3%; Table 4; *P*=.0025). Presumed fertile patients exhibited a significantly higher implantation rate of euploid blastocysts in a corresponding FET cycle when compared with infertile patients, 36/42 (85.7%) and 54/109 (66.7%), respectively (Table 4; *P*=.0001). There was no difference between the number of multiples or spontaneous abortions between presumed fertile and infertile patients (Table 4).

TABLE 3

Primary diagnosis of patients undergoing an FET cycle.

	Presumed fertile	Infertile
No. of patients	31	62
No. of frozen ET cycles	33	81
No. of recurrent pregnancy loss (%)	—	17 (21.0)
No. of primary infertility (%)	—	1 (12.4)
No. of secondary infertility (%)	—	3 (3.7%)
No. of polycystic ovarian syndrome (%)	—	9 (11.1)
No. of diminished ovarian reserve (%)	—	8 (9.9)
No. of uterine factor (%)	—	3 (3.7%)
No. of severe male factor (%)	—	13 (16.1)
No. of unexplained (%)	—	15 (18.5%)
No. of advanced maternal age (%)	—	7 (8.6)
No. of endometriosis (%)	—	5 (6.2)
No. of single gene (%)	10 (30.3)	—
No. of sex selection (%)	8 (24.2)	—
No. of anonymous egg donor (%)	15 (45.5)	—

Taylor. Fertile and infertile implantation rates. Fertil Steril 2014.

DISCUSSION

Our data do not support our hypothesis that infertile patients exhibit a higher rate of aneuploidy than age-matched presumed fertile patients (Tables 1 and 2). However, our data do support our hypothesis that euploid blastocysts derived from infertile couples have significantly lower implantation potential when compared with euploid blastocysts from presumed fertile couples (Table 4).

In both age groups, no statistical difference in aneuploidy rates was seen between presumed fertile and infertile patients. The literature is limited concerning the rate of aneuploidy in fertile women ≥35 years as this patient group typically does not resort to IVF to achieve pregnancy. Ata and colleagues (17) described a direct relationship between aneuploidy and maternal age, indicating that approximately 30% of blastocysts from oocyte donors are aneuploid, while approximately 80% of blastocysts from women ≥43 years old are aneuploid. In our study, we found that approximately 40% of blastocysts produced by presumed fertile patients (which included oocyte donors) <35 years old were aneuploid, which is similar to the rate of aneuploidy described in donor oocyte cycles.

Fragouli and colleagues (18) used comparative genomic hybridization and examined the polar bodies from young donors. They found a low aneuploidy rate of 3%. In contrast, some reports indicate aneuploidy rates in polar bodies derived from donors as high as 65% (19). It is important to examine aneuploidy rates in an older, presumed fertile patient population because individuals achieve pregnancy in their late 30s and early 40s without the aid of IVF. When subdivided by age, our data demonstrated that regardless of whether a patient is fertile or infertile, the incidence of preimplantation aneuploidy is similar.

Fragouli and colleagues (18) also hypothesize that during a natural cycle in a normal, proper functioning ovary, aneuploid oocytes may be selected against. However, controlled ovarian hyperstimulation or aging may circumvent this mechanism. Presumably, fertile patients have properly functioning ovaries that are exposed to hyperstimulation drugs.

TABLE 4

Outcomes in frozen ET cycles of euploid blastocysts in presumed fertile and infertile patients.

	Presumed fertile	Infertile	*P* value
No. of patients	31	62	
No. of frozen ET cycles	33	81	
Maternal age at retrieval ± SD	34.2 ± 3.8	35.5 ± 3.6	.1168[a]
No. of blastocysts thawed	43	109	
No. of blastocysts survived (%)	42 (97.8)	109 (100.0)	.2829[b]
Average no. transferred ± SD	1.3 ± 0.5	1.4 ± 0.5	.5695[a]
No. transferred	42	109	
Good-quality blastocysts (%)	12 (28.6)	20 (18.4)	.3790[c]
Fair-quality blastocysts (%)	28 (66.7)	82 (75.2)	
Poor-quality blastocysts (%)	2 (4.8)	7 (6.4)	
+HCG (%) per transfer	28 (84.8)	50 (61.7)	.0251[b]
+Sac (%) per transfer	28 (84.8)	44 (54.3)	.0025[b]
No. of sacs (% implantation)	36 (85.7)	54 (66.7)	.0001[c]
No. of multiples per pregnancy (%)	8 (24.2)	10 (20.0)	.5607[c]
Spontaneous abortions per pregnancy (%)	3 (8.3)	2 (4.0)	.3436[b]
Average no. of euploid available	3.4 ± 1.7	2.6 ± 1.6	.0154[a]

[a] Mann-Whitney *U*-test.
[b] Fisher's exact test.
[c] χ^2-test for independence.

Taylor. Fertile and infertile implantation rates. Fertil Steril 2014.

Therefore, regardless of whether a patient is fertile or not, the hyperstimulation of ovaries produces similar aneuploidy rates. In our study, presumed fertile patients <35 years old had significantly higher euploid blastocyst rates (59.3%; Table 1) when compared with presumed fertile women ≥35 years old (42.9%; Table 2). The same trend was seen when euploid blastocyst rates were compared between infertile patients <35 years old (56.3%; Table 1) and ≥35 years old (42.2%; Table 2). Therefore, our data suggest that age and not hyperstimulation undermines this mechanism. Further evidence of this is the fact that aneuploidy is present in embryos derived from nonstimulated ovaries (20).

Other studies have reported similar pregnancy rates between fertile and infertile women when only euploid blastocysts were transferred during FET cycles (21). Pregnancy rates from euploid blastocysts have been reported to range from 65% to 70% (22). Our overall pregnancy rates are similar to those in this report (68.4%); however, we also observed significantly higher pregnancy and implantation rates in the presumed fertile patients compared to infertile patients. This would indicate that a factor other than chromosomes is hindering pregnancy in infertile patients, such as an increased amount of mitochondrial activity, altered gene expression, or possible uterine factors (23, 24). One possible explanation for the increase in implantation rates seen with presumed fertile patients is the fact that they had more blastocysts to biopsy when compared with infertile patients (Table 1). With more blastocysts to biopsy and vitrify, these patients would have more euploid blastocysts to choose from in the corresponding FET cycle. This assumption is correct, however, when embryo quality before warming is examined, there is no difference between presumed fertile and infertile patients (Table 4). Lastly, a majority of presumed fertile patients underwent PGS for either sex selection or single-gene disorders. Therefore, the sex of the embryo or the single-gene diagnosis, not embryo quality, was used as the first line of selection in this group of patients.

Lastly, our data are retrospective and require an understanding of presumed fertile and infertile. There are many diagnoses that may include or exclude patients from these categories and possibly influence our results. Presumed fertile patients were only those who desired IVF for either gender selection, single-gene disorder, or anonymous oocyte donation. Theoretically these patients do not require IVF to achieve pregnancy. Infertile patients encompass a much larger spectrum of diagnosis; however, all these patients underwent IVF to achieve pregnancy. For example, only patients with severe male factor (<1 million per mL) were included in the infertile group. Moreover, secondary infertility includes patients who have exhibited previous fertility but have not been successful after a full year of unprotected intercourse. Possible reasons for secondary infertility include increased maternal age and weight gain. In our study, all of our secondary infertility patients had a secondary diagnosis as well (advanced maternal age, ovulation disorder, or unexplained infertility). Lastly, maternal age is the single largest factor affecting chromosomal aneuploidy and IVF success rates (25). Patients with advanced maternal age (AMA) were included in our study. By definition, those patients who were presumed fertile and ≥35 years old could be diagnosed with AMA, however, they did not need IVF to achieve pregnancy. Infertile patients diagnosed with AMA were undergoing IVF specifically because they could not conceive naturally. Our presumed fertile group was not undergoing IVF to conceive but rather to select a child with certain characteristics. The infertile group was undergoing IVF specifically because they could not achieve a successful pregnancy.

In conclusion, our data suggest that blastocyst euploidy rates are similar, regardless of whether a patient is presumed fertile or infertile. Although our numbers are small, presumed fertile patients show a significantly higher pregnancy and

implantation rate compared with infertile patients when a euploid blastocyst is transferred during an FET cycle. This would indicate that infertility as a disease may encompass other aspects such as uterine or other unknown embryological factors that can influence outcomes. Further studies are needed to determine these factors.

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