IMPACT STATEMENT: While the application of FET has become popular due to its proven safety, the addition of embryo ploidy testing helps to normalize clinical outcomes in cases of advancing female partner age.

SUPPORT: None

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ENDOMETRIAL PREPARATION BY HORMONE REPLACEMENT AND DAY 6 BIOPSIED EUPLOID BLASTOCYST TRANSFER (BT) INCREASES THE RISK OF MISCARRIAGE COMPARED WITH AN OVULATORY CYCLE AND DAY 5 BIOPSIED EUPLOID BT.

Takashi Horikawa, MD, Koji Nakagawa, M.D., PH.D., Hideaki Watanabe, Ph.D., Keiji Kuroda, MD, PhD, Satoru Takamizawa, MD, PhD, Rikikazu Sugiyama, MD, PhD Sugiyama Clinic Shinjuku, Tokyo, Japan.

OBJECTIVE: Accepted theory posits that more than half of the causes of pregnancy loss involve aneuploidy of the transferred embryos or blastocysts. This explanation has recently come into question, however, due to the clinical application of preimplantation genetic testing for aneuploidy (PGT-A). In an effort to further elucidate the risk factors for miscarriage, we retrospectively evaluated the clinical data between ongoing pregnancies and cases of miscarriage following euploid blastocyst transfer.

MATERIALS AND METHODS: Between January 2020 and April 2021 we evaluated 389 pregnant women who had received euploid blastocyst transfer. Written informed consent was obtained from each participant. All blastocysts underwent a TE biopsy on either Day 5 (D5) or Day 6 (D6) for PGT-A, and those were cryopreserved. The extracted TE cells were analyzed via NGS. The endometrial preparation for thawed euploid blastocyst transfer was accomplished either by hormone replacement cycle (HRC) or natural ovulatory cycle (NOC). Clinical pregnancy was defined as the existence of a gestational sac via transvaginal ultrasound. The miscarriage rate was evaluated according to the clinical background. A p-value of <0.01 was considered statistically significant. All statistical analyses were performed with EZR.

RESULTS: Among 389 pregnant women, 42 miscarried for a miscarriage rate (MR) of 10.8%. According to the endometrial preparation, HRC showed a higher MR (16.0%) than NOC (7.5%, $p<0.01$). D6 biopsy and repeated implantation failure (RIF) cases also showed a higher MR than that shown by D5 biopsy (17.3% vs. 9.1%, $p<0.05$) and by non-RIF cases (18.5% vs. 6.7%, $p<0.01$). However, there was no difference between the MR in the cases of repeated pregnancy loss (PRL) (9.2%) and that in the non-PRL cases (11.6%, $p=0.487$).

CONCLUSIONS: From this evaluation using euploid blastocyst transfer, HRC showed an MR that was higher than that for NOC. It seemed that no formation of corpus luteum in the HRC could lower the MR. Moreover, the extended culture needed for TE biopsy could cause the cytoplasm of embryos to age, and this could be a reason for the lower MR of the D6 blastocysts.

IMPACT STATEMENT: This study suggests that practitioners should either use a method to select the best blastocyst from among euploid blastocysts or use an endometrial preparation method.

SUPPORT: none

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COMPARISON OF CLINICAL OUTCOMES IN THE SLOW-DEVELOPING BLASTOCYSTS WITH OR WITHOUT PREIMPLANTATION GENETIC TESTING-ANEUPLOIDY (PGT-A) IN THE FROZEN-THAWED CYCLE.

Jihye Jeong, M.S., Juhee Lee, M.S., S. O. L. A. Yoon, B.S., Jisoo Han, PhD, Tae Hyung Kim, B.S., Jin Hee Eum, Ph.D., Ji Eun Park, M.D., Haengjun Jeon, M.D., Jin Yeong Kim, M.D. Ph.D, Woo Sik Lee, M.D. Ph.D. CHA Gangnam Medical Center, CHA University, Seoul, Korea, Republic of (South).

OBJECTIVE: Preimplantation genetic testing (PGT) is used for selecting euploid embryos to improve pregnancy outcomes after embryo transfer. Several research studies have suggested that slow-developing blastocysts on day6 show lower implantation rates (IR) compared to normal developing embryos on day5. However, some of researchers focus on that slow developing embryos are highly derived from women with diminished ovarian reserve. Therefore, this study was performed to investigate the potential of the slow-developing blastocysts with PGT-A in patients with recurrent spontaneous abortion (RSA) and/or recurrent implantation failure (RIF) undergoing FET.

MATERIALS AND METHODS: A total of 127 FET cycles with single embryo transfer (SET) were evaluated. This study evaluated the slow-developing blastocysts with or without PGT-A in couples with RSA and/or RIF undergoing FET from January 2020 to January 2022. 90 patients underwent freeze on day6, and 37 patients underwent freeze after embryo biopsy on day 6, respectively.

RESULTS: Female age was not different (36.1±3.5 yr vs. 36.7±3.6 yr, $p=0.19$) in the two groups. The clinical pregnancy rate and miscarriage rate were analyzed in these two groups. The clinical pregnancy rate (CPR, detectable gestational sac in 6th week of gestation) in the biopsy group was higher than that in the non- biopsy group, but there was no statistical significance (56.8% vs 52.2%, $p=0.64$). Otherwise, the miscarriage rate in the non-biopsy group (31.9%) was significantly higher than that in the biopsy group on day6 (4.8%, $p=0.051$).

CONCLUSIONS: The present study demonstrate that the biopsy group on day 6 showed higher clinical pregnancy rate and lower miscarriage rate than those in the non-biopsy group on day 6.

IMPACT STATEMENT: Our data support that the selecting day6 blastocysts using PGT-A for transfer may reduce the risk of miscarriage and maintain pregnancy in women with RSA and/or RIF.

SUPPORT: This research was supported by a grant from Republic of Korea, NRF-2018R1D1A1B07043250.

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CLINICAL AND EMBRYOLOGICAL FACTORS ASSOCIATED WITH A HIGH PROPORTION OF MOSAIC EMBRYOS: CAN WE IDENTIFY ANYONE AT RISK?

Chelsea M. Canon, MD,[1] Teresa Cacchione, MS, CGC,[2] Dmitry Gounko, B.S., M.A.,[2] Carlos Hernandez-Nieto, MD,[2] Joseph A. Lee, BA,[2] Beth McAvey, M.D., M.S.,[3] Daniel E. Stein, MD,[2] Lucky Sekhon, MD,[2] Alan B. Copperman, MD[2] [1]Icahn School of Medicine at Mount Sinai, New York, NY; [2]Reproductive Medicine Associates of New York, New York, NY; [3]Reproductive Medicine Associates Long Island IVF (RMALIIVF), Garden City, NY.

OBJECTIVE: Mosaicism is defined as the presence of more than one cell line in a single embryo and is inferred on preimplantation genetic testing for aneuploidy (PGT-A) by an intermediate copy number on next generation sequencing (NGS). The estimated rate of reported mosaicism is between 3-20%[1]. However, there are selected patients who experience higher proportions of mosaic embryos than expected compared to the general patient population who undergoes in vitro fertilization (IVF) with PGT-A. The objective of this study was to investigate whether there are risk factors associated with patients who have an increased proportion of embryos reported to be mosaic in a single IVF with PGT-A cycle.

MATERIALS AND METHODS: This study included all patients undergoing IVF with intracytoplasmic sperm injection (ICSI) and PGT-A at a single academic center from January 2020-March 2022. All PGT-A testing was performed using next generation sequencing (NGS) that reported for mosaicism. Our primary outcome was mosaicism rate. Cycles were categorized by the percentage of embryos diagnosed on PGT-A as mosaic (as $\leq$ 20% or $>$20%) and demographic and cycle characteristics were collected. Comparative statistics were performed with t-test, Kruskal-Wallis, and chi-square. The groups were analyzed using a multivariate regression analysis.

RESULTS: A total of 3,848 IVF cycles were included in our analysis. 17,505 embryos were biopsied with a 10.4% average rate of mosaicism. Baseline characteristics were obtained and examined including: primary diagnosis, oocyte age, partner age, semen analysis parameters, body mass index (BMI), anti mullerian hormone (AMH), basal antral follicle count, Day 3 follicle stimulating hormone, estrogen at time of surge, cumulative and individual gonadotropin dose (recombinant follicle stimulating hormone/menotropin/combined). After multivariate regression analysis, no patient or cycle characteristics were found to be significantly associated with increased odds of $>$20% mosaicism in an embryo cohort from a single cycle.

CONCLUSIONS: This study was unable to identify any patient or cycle characteristics that are associated with having a high proportion of mosaic embryos from IVF with PGT-A. Although we have previously reported that older oocytes are more likely to result in individual embryos being classified as aneuploid rather than mosaic, our findings are in agreement with the literature that states there are no known risk factors that are correlated with

IVF cycles that have a high percentage of embryos classified as mosaic. Further research into the mechanism behind post-fertilization mitotic errors and predisposing risk factors is needed.

IMPACT STATEMENT: Patients can be assured that there is no individual and/or cycle-specific characteristic found to be associated with experiencing >20% embryos diagnosed as mosaic in a single IVF with PGT-A cycle.

SUPPORT: None

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ASSESSING PATIENT COMPLIANCE WITH RECOMMENDED PRENATAL TESTING AND IDENTIFYING PREGNANCY AND NEONATAL OUTCOMES AFTER MOSAIC EMBRYO TRANSFER.

Stephanie M. Hallisey, MD,[1] Katherine Koniares, MD,[1] Arti Taggar, M.D., M.P.H,[1] Prachi N. Godiwala, MD,[2] Daniel R. Grow, MD, MHCM[1] [1]University of Connecticut Health Center, Center for Advanced Reproductive Services, Farmington, CT; [2]Center for Advanced Reproductive Services, Farmington, CT.

OBJECTIVE: Preimplantation genetic testing for aneuploidy (PGT-A) is used to aid in selecting optimal embryos for transfer after in vitro fertilization (IVF) cycles. There is building evidence that transferring mosaic embryos can lead to live births clinically unaffected by chromosomal abnormalities. Prenatal testing with an amniocentesis is recommended after mosaic embryo transfer due to the possibility of placental mosaicism. Though this recommendation exists, no studies have evaluated patient compliance. Likewise, limited studies have evaluated the effect mosaicism may have on pregnancy outcomes and neonatal/infant syndromes after transfer. Therefore, our objective was to assess patient compliance with recommended prenatal amniocentesis following mosaic embryo transfer and to investigate the effect of mosaic embryo transfer on pregnancy and neonatal outcomes.

MATERIALS AND METHODS: This case series included 21 mosaic embryo transfers from 19 women aged ≤45 years who underwent IVF with PGT-A. Single mosaic frozen embryo transfers performed between 5/2017 and 3/2021 at a single academic fertility center were included. Data collection was performed via chart review and telephone interview of patients. The primary outcome was performance of prenatal amniocentesis. Secondary outcomes included alternative prenatal genetic testing, pregnancy or delivery complications and diagnosed neonatal/infantile medical conditions.

RESULTS: Of the 21 mosaic embryo transfers included in the study, 14 were low-level mosaic (67%) and 7 were high-level mosaic (33%). Four transfers did not result in pregnancy (19%), four resulted in biochemical pregnancy (19%), one resulted in miscarriage (5%), one resulted in an ongoing pregnancy (5%), and eleven resulted in live births (52%). Of the 12 ongoing pregnancies/live births, only 2 prenatal amniocenteses were performed (16.7%). Both amniocenteses were negative for all conditions tested. Chorionic villus sampling was not performed after any transfer. Non-invasive prenatal testing with cell-free DNA was performed in 6 cases and was negative for all conditions tested. A postnatal karyotype was performed in one case with findings of a balanced translocation consistent with the paternal karyotype. There were no pregnancy or delivery complications. There was one case of Tetralogy of Fallot identified prenatally with no underlying chromosomal abnormality found. There were no other neonatal/infantile medical conditions identified.

CONCLUSIONS: Most patients achieving pregnancy after a mosaic embryo transfer did not undergo recommended prenatal amniocentesis testing. Of those who did, the results were negative for all tested conditions. There were no pregnancy or delivery complications, or neonatal/infantile medical conditions identified after transfer.

IMPACT STATEMENT: Patient compliance with prenatal amniocentesis following mosaic embryo transfer is low. To address this, we recommend initiating the use of clinical educational protocols and specific consent forms for mosaic embryo transfer to emphasize the recommendations for genetic testing.

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THE PERFORMANCE AND IMPLICATION OF TWO WHOLE GENOME AMPLIFICATION APPROACHES FOR NON-INVASIVE GENETIC TESTING.

Zhixin Hu, B.S.,[1] Yonggang Li, M.S.,[2] Lifeng Xiang, Ph.D,[2] Kexin Chen, B.S.,[1] Mei Tian, M.D.,[2] Mingying Li, M.SC.,[2] Yunxiu Li, M.D.,[2] Xi Luo, M.D.,[2] Xiaorong Wu, M.D.,[2] Ze Wu, Ph.D,[2] Jiacong Yan, Ph.D[2] [1]Kunming University of Science and Technology School of Medicine, Kunming, China; [2]Department of Reproductive Medicine, First People's Hospital of Yunnan Province, Kunming, China.

OBJECTIVE: To investigate the performance and differences of two whole-genome amplification (WGA) methods of Malbac and PicoPLEX in non-invasive preimplantation genetic testing(niPGT) based on spent media (SCM) in PGT cycle.

MATERIALS AND METHODS: From 2020∼2022, 122 embryos from PGT-SR patients and 61 embryos from PGT-A patients were tested with invasive biopsy based NGS as golden standard. All embryos underwent laser-assisted hatching incubation on Day4. Trophoectoderm(TE) cells were collected from each embryo on day 5-6 and detected by MALBAC-NGS. SCM samples(30μl) were collected at Day5/6, and a single SCM sample was divided into two aliquots using two WGA methods, noninvasive genetic testing(MALBAC,Yikon) or DOP-PCR(PicoPLEX,Agene). All SCM samples were from fresh embryos. For TE biopsy, >4Mb copy number variation(CNV) and 30∼80% mosaic ratio is considered. For SCM,>10Mb CNV and 30∼80% mosaic ratio is considered.

RESULTS: In the whole PGT, SCM-DOP-PCR and SCM-MALBAC had a higher karyotype concordance rate of 79.03%(49/62) and 82.1%(55/67) for apparently abnormal (abnormal fragments > 20M) TE biopsy. However, for PGT-SR, SCM-DOP-PCR and SCM-MALBAC samples showed 66.7%(60/90) and 69.3%(61/88) clinical concordance with the corresponding TE biopsy samples. For PGT-A, SCM-DOP-PCR and SCM-MALBAC samples showed 51.2%(21/41) and 53%(25/46) clinical concordance with the corresponding TE biopsy samples. Additionally, no statistically significant differences were detected in the aforementioned values of the SCM-DOP-PCR and SCM-MALBAC in either clinical concordance rate or apparent abnormal concordance rate ($P > 0.05$). In addition, the amplification rates of DOP-PCR and MALBAC were 77.6%(142/183) and 77.05%(141/183), respectively. Though both methods show higher abnormal chromosome rate with differences region, no significant hotspot observed. In general, there are some differences between SCM and biopsy, for which we focused on follow-up. The current 4 follow-up results showed that the prenatal diagnosis was consistent with the TE biopsy, even in the miscarriage samples.

CONCLUSIONS: Our result suggest that there seems no significant difference(including amplification success rate, clinical consistency and many more) between DOP-PCR and MALBAC in niPGT studies. However, the high karyotype concordance rate of niPGT and biopsy in clearly abnormal fragments will have certain application prospects.

IMPACT STATEMENT: Two different detection approach with different bias suggest the technic details of non-invasive testing requires improvement currently. More basic and clinic research are required to promote the application of this technology. Combining biopsy based approach and non-invasive approach may be considered as a comprehensive tools for embryo implantation prediction. At the same time, the current research results can prove that niPGT is a good choice in excluding obviously abnormal embryos for people who do not meet the subclinical criteria for PGT application.

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IMPACT OF DISCREPANT VARIANT CLASSIFICATION ON PREIMPLANTATION GENETIC TESTING FOR MONOGENIC CONDITIONS (PGT-M).

Rawan I. Awwad, MS, CGC,[1] Andria G. Besser, MS, CGC,[2] James A. Grifo, MD, PhD,[3] Gina M. Davis, MS, CGC[4] [1]Certified Genetic Counselor, Birmingham, MI; [2]NYU Langone Fertility Center, New York, NY; [3]NYU Langone Prelude Fertility Center, New York, NY; [4]Advocate Genetics, Elk Grove, CA.