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Spring 2023, Volume 8, Issue 29 (1-7)

Maternal Age-Related Chromosomal Aneuploidy in Human Day 3 Embryo Biopsy Blastomeres in Couples Undergoing IVF Cycles in Kuwait

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DOI: 10.22034/pmj.2023.705457

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Submitted: 2023-03-25
Accepted: 2023-05-21

Keywords:
Next Generation Sequencing
Genetics
Blastomeres
Aneuploidy
Chromosomes

Abstract:
Objectives: Next Generation Sequencing (NGS) has been a popular platform for preimplantation genetic testing of embryonic aneuploidy. The main objective is to study the rate of aneuploidy in human blastomeres at day 3 embryonic division biopsy in Kuwait using NGS and to further assess the relationship between maternal age and the complexity of embryonic chromosomal aneuploidy.
Methods: Data was collected from a single genetics centre in Kuwait through electronic records. This retrospective study of 101 patient covered a period of 4 months (January 2022- April 2022). Information on chromosomal errors were stratified using contingency tables. Statistical analysis of aneuploid outcomes was carried out to estimate value between different maternal age groups. P values under 0.05 were considered statistically significant.
Result: A total of 808 blastomeres were included in the study and deemed to have met our inclusion criteria. There was a total of 572 (70.8%) aneuploid blastomeres. Highest percentage of aneuploidy was within embryos collected from maternal ages 40 years and above (72.7%). A total of 686 aberrations were within chromosomes 13, 18, 21, X, and Y, 30.3% of which were an aneuploidy within chromosome X. Majority (66.8%) of blastomeres demonstrated an aberration in at least 3 of its chromosomes and therefore deemed as complex. Overall trisomy to monosomy ratio was 3.169.
Conclusion: There was a wide-ranging variability regarding mean number of blastomeres examined across the different maternal ages. Our study has reflected the well-known association of embryonic aneuploidy with increased maternal age given the highest proportion of error (72.6%) was within blastomeres from maternal ages 40 years and above. Overall statistical analysis has however demonstrated no significant difference regarding rate and complexity of aneuploidy in blastomeres across the different maternal age groups. Larger cohort studies of aneuploidy are therefore needed for further evaluation and improve patient counselling.

INTRODUCTION

Aneuploidy refers to the presence of an abnormal number of chromosomes in a cell that traditionally takes place with the occurrence of an extra or missing chromosome (1). Aneuploidy therefore allows cells to form an unbalanced chromosome complement. Preimplantation genetic diagnosis (PGD) has been known as an effective approach for identifying aneuploidy and selecting best quality euploid embryos for transfer in patients undergoing In Vitro Fertilisation (IVF) treatment. The main principle underlying PGD testing in this circumstance is excluding embryos with abnormal chromosomes in the hopes of a better chance of a successful pregnancy (2). This mode of testing is utilised globally, particularly in cases of advanced maternal age, repeated miscarriages, and repeated implantation failure (2). Earlier methods for preimplantation genetic testing included the use of fluorescent in situ hybridisation (FISH). FISH was quick and sensitive in the identification of chromosomes at the time, nevertheless, disadvantages such as split and overlapping signals have been well documented (3). Different techniques of embryo selection were developed after years of advancements in analysis methods for preimplantation genetic testing for aneuploidy as to improve resolution and reduce

costs. Next Generation Sequencing (NGS) has been the popular platform for preimplantation genetic testing of Aneuploidy (PGT-A) since 2015, replacing the well-known comparative genomic hybridisation microarray (aCGH) and FISH (3). Development of such testing platforms and advances in laboratory practice have resulted in improvements in IVF outcomes over the past twenty years and empowered the assessment of the ploidy status of embryos before transfer (4). Age-related embryonic aneuploidy nonetheless remains as a clear issue in realising a fully successful IVF process. This association between maternal age and Aneuploidy has been firmly established in previous literatures (5,6). There, however, appears to be a lack of consensus regarding the rate of age-related aneuploidy in the State of Kuwait. This study will allow us to study this association and provide an opportunity to consider the nature of the aneuploidies identified.

OBJECTIVE

The main objective is to study the prevalence of aneuploidy in human blastomeres at day 3 embryonic division biopsy in a sample cohort in Kuwait using Next Generation Sequencing Technique (NGS). This study will also allow us to further evaluate and characterise the relationship between the nature of the human embryonic chromosomal aneuploidy and age of the female partner, including number of chromosome involvement and ratio of Trisomy to Monosomy.

MATERIALS AND METHODS

Study design and participants (patient group)

In this retrospective study, data was collected over a 4-month period (January 2022- April 2022) from a single general practice and genetics centre within Al-Ahmadi district, Kuwait. Information on 101 patients were gathered from an electronic medical database and stored in a self-composed excel sheet. Primary analysis was based on the determination of the percentage of blastomeres that were aneuploid in relation to the age of the female partner (based on age group). Information on patient details, age, number of blastomeres examined and characteristics of the chromosomal aberrations present within each blastomere was collected. Information on aneuploid rates and number of errors were stratified across the different age groups using contingency tables and statistical analysis. Out of the total 101 patients, 6 cases were excluded since the embryos examined originated from parents with a known translocation. Cased with incomplete date were also excluded from the study.

Sequencing modality and Whole Genome Amplification

Human blastomeres samples were collected from IVF centres around Kuwait. Analysis was done using Thermofisher Ion Reporter™ software 5.20. For Library Preparation, gDNA was extracted and amplified using the Reproseq™ PGS Kit. Ion SingleSeq Barcodes were added to each sample accordingly. Pooled purified library was quantified using the Qubit dsDNA HS (High Sensitivity) assay kit and the Qubit 2.0 Fluorometer to finalise the dilution of pooled purified library. With regards to template Preparation (Reproductive Ion ReproSeq Aneuploidy template), runs were created on Torrent server using TorrentSuite Software. The purified library finally loaded to IonChef Instrument. Sequencing was carried out with the Ion Gene Studio S5 Prime Instrument preceded with initialization. Analysis was successfully achieved after uploading samples data to Thermofisher Ion ReporterTM software 5.20 account for results to be analysed.

STATISTICAL ANALYSIS

Statistical analysis was carried out using Stata 17. Patient data was analysed using Fisher's exact test for comparison of data primarily categorised based on patient age, number of aneuploid embryos and nature of chromosomal aberrations present. Null hypothesis in this article claims that there was no significant difference in percentage of aneuploidy between the different age groups of the female partner. Equality of group means test (Wilks's lambda) was used to estimate value between different maternal age groups. Two sample T tests were utilised for estimation of significance of results between maternal ages under 40 and those above. *P* values under 0.05 were considered statistically significant. Analysis of outcomes and confidence intervals were constructed sing computerised standard estimates allowing us to compare degree of aneuploidy across different age groups.

ETHICS

This study was approved and registered by the centre's institutional review board in Kuwait. All patient details and data were anonymised before analysis. No informed written consent was required. This project did not require an application for Caldicott approval.

RESULTS

Participant and patient characteristics (demographic and clinical information)

95 patients in total were identified as fitting our inclusion criteria and therefore included in the study. As aforementioned, 6 cases were excluded since the embryos examined had originated from parents with a known translocation. Cases with incomplete date were also excluded from the study. All blastomeres examined were at day 3 embryonic division. A total of 808 blastomeres were included in the study. Ages

of the female partner ranged from 20 years to 49 years and divided into 5 groups. Figure 1 shows the mean, median, maximum, and minimum number of blastomeres examined according to all maternal ages included in our article. Our study demonstrated a wide ranging variability in regard to mean number of blastomeres examined across the different maternal ages with the median number exceeding 10 blastomeres only from maternal ages 28, 33, and 36 years. Most blastomeres samples were from group 4 (maternal ages 35-39 years), making about 38.4% of the total studied blastomere sample. Case demographics, results for aneuploidy and nature of chromosomal aberrations across each age group are shown in Table 1.

Euploid and Aneuploidy outcomes

There were 808 stage 3 blastomeres collected from a total of 95 patients. From the sample, there was a

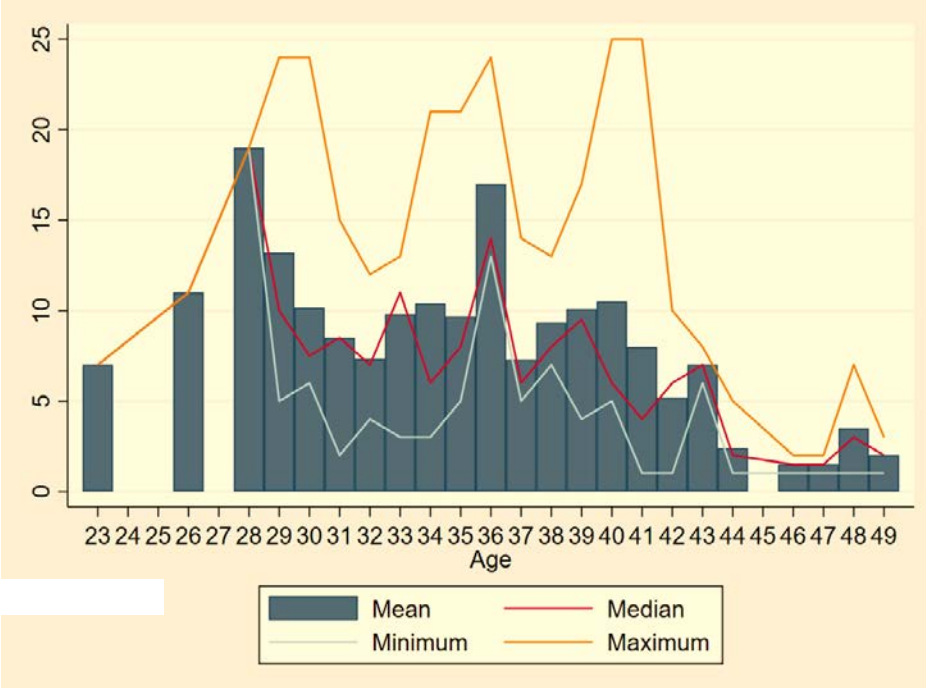

Fig1. Overall descriptive statistics of stage 3 blastomeres examined according to maternal age.

Table 1. Distribution of blastomeres, results for aneuploidy and nature of chromosomal aberration present (if any) across the different maternal age groups.

Age Grp		No of Blasto-mere	Euploid		Aneuploid		No. of Chromosomal Errors			
Group	Age Range		(n)	% of total	(n)	% of total	Single Error	Dual Error	Multiply Error (3+)	Trisomy : Monosomy
Group 1	20-24	7	2	28.5	5	71.4	0 (0%)	0 (0%)	5 (100%)	1.235
Group 2	25-29	96	27	28.1	69	71.8	13 (18.8%)	8 (11.6%)	48 (69.6%)	3.514
Group 3	30-34	223	68	30.5	155	69.5	29 (18.7%)	27 (17.4%)	99 (63.9%)	3.302
Group 4	35-39	310	92	29.6	218	70.3	44 (20.2%)	31 (14.2%)	143 (65.6%)	3.076
Group 5	40+	172	47	27.3	125	72.7	21 (16.8%)	17 (13.6%)	87 (69.6%)	3.142
Total		808	236		572		107	83		

total of 236 (29.2%) blastomeres with a fully balanced chromosomal complement and hence deemed as euploid. There was a total of 572 aneuploid blastomeres making about 70.8% of the studied population. The mean number of aneuploidies according to age of all female partners in the study is shown on figure 2. Our study showed no significant difference with the regards to aneuploidy in stage 3 blastomeres across the different age groups. P-value 0.9675. Highest rate of aneuploidy was within group 5 (those aged 40 years and above) making about 72.7% of the total number of blastomeres collected from this demographic. 155 blastomeres examined from group 3 (maternal ages 30-34 years) demonstrated a form of aberration across at least one the of the chromosomes, which approximately correlates to about 69.5% of this cohort. This group presented with the lowest fraction of aneuploidy for stage 3 blastomeres and consequently the highest proportion of euploid blastomeres within its cohort (30.5%).

Further analysis of chromosomes 13, 18, 21, X (Chromosome 23), and Y (Chromosome 24)

In this article, further analysis took place in place to study and categorise aberrations by chromosome number, more specifically chromosomes 13, 18, 21, X (Chromosome 23), and Y (Chromosome 24). Out of the 808 blastomeres, there was a total of 686 aberrations within the five chromosomes, most of which were an aneuploidy within chromosome X, forming about 30.3 % of the documented total. Least number of aberrations, irrespective of patient age, were in Chromosome Y (Chromosome 24) making 5.25% of the total number of aberrations. There was no significant difference regarding percentage of aneuploidy within those five chromosomes between the different age groups (groups 1 -5). P-value 0.9950. Further stratification and grouping of blastomeres into two age groups (maternal ages under and those equal to and above 40 years) have also shown no significant difference in proportion of aneuploidy for those 5 chromosomes between the two groups. P value 0.8322 (95% Confidence interval 0.766 -0.932 for the combined total).

Complexity of Aneuploidy and ratio of Trisomy to Monosomy

As previously mentioned, a total of 572 blastomeres exhibited an aneuploidy in at least one of its chromosomes making about 70.8% of the total number examined. Blastomeres were further characterised by complexity of the aneuploidy present, that is, either involving one, two, or three or more chromosomes. A total of 382 blastomeres demonstrated an aberration in at least 3 of its chromosomes making about 66.8%, and hence the majority, of the total sample examined. There were 107 blastomeres (18.7%) that were aneuploid for just a single chromosome within the total sample of examined blastomeres. Errors involving two chromosomes were least common, making about 14.5% of the total sample. Figure 3 further demonstrates nature and complexity of aneuploidy across the maternal ages involved in this article. Errors involving three or more chromosomes exceeded 60% of mean result in maternal ages 23, 42, 44, 46, 47, and 48 years, forming about 69.6% of the total number of chromosomal aberrations in group 5 and a 100% of that in group 1. Our study showed no significant difference between complex aneuploidy (involving three or more

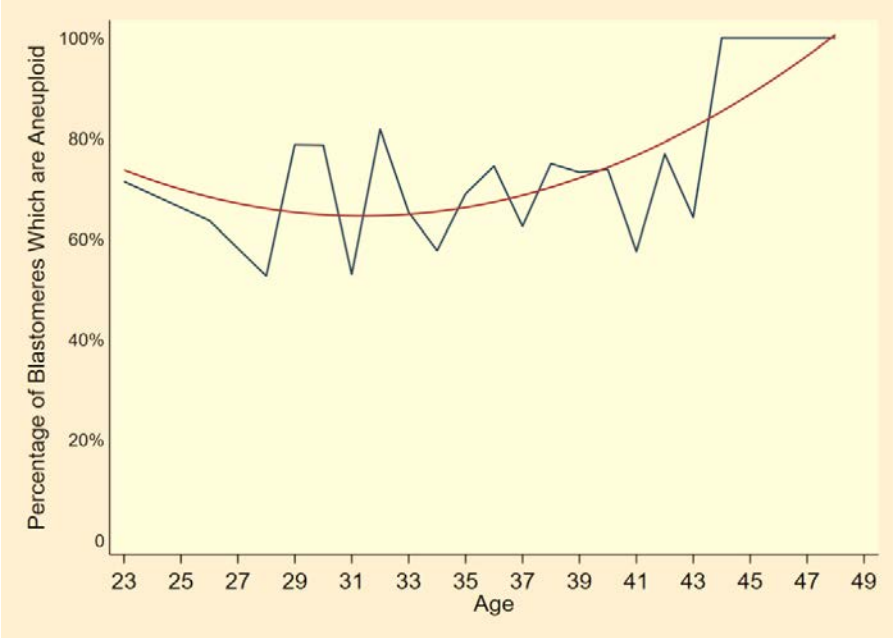

Fig2. The percentage of blastomere aneuploidy per age of female partner across the study.

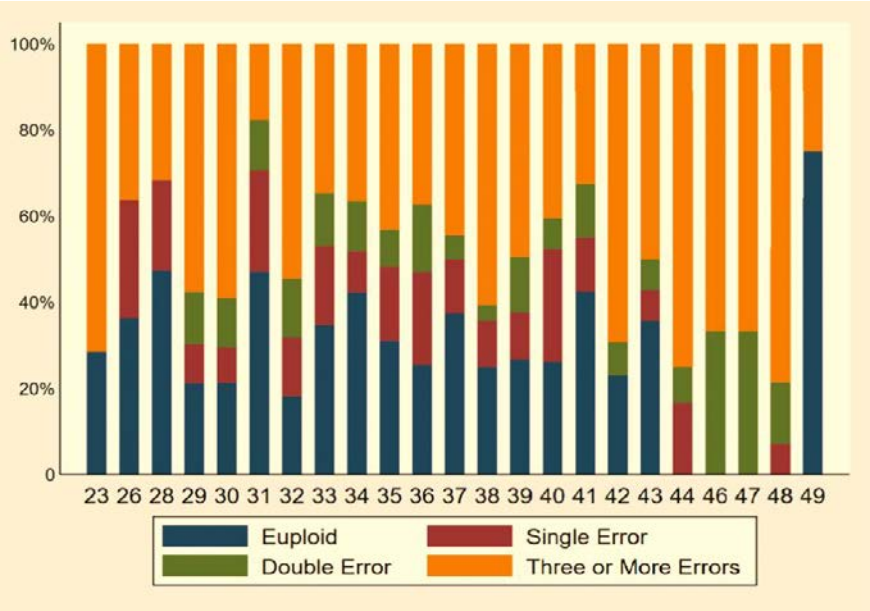

Fig3. Complexity of blastomere chromosomal aneuploidy per age of female partner involved in the study.

chromosomes) and age of female partner across all age groups (1-5). P value 0.4666. Same conclusion (no significant difference) was inferred for simpler errors involving one or two chromosomes across the different age groups.

Trisomy and Monosomy aberrations formed the two main types of errors our study. Among the 808 blastomeres examined, there were a total 3956 errors identified, 3007 were a result of Trisomy and 949 a result of Monosomy. Overall, this results in a Trisomy to Monosomy ratio of 3.169. Highest ratio of Trisomy to Monosomy errors were within group 2 (maternal ages 25-29) with a ratio of 3.514. This was a sharp contrast to the sample collected from group 1 (maternal ages 20-24) who've had a ratio of 1.235. Figure 4 illustrates the relationship between maternal age and the mean Trisomy: Monosomy ratio within the total blastomere sample.

DISCUSSION

The present study assessed estimates of aneuploidy in day 3 embryonic division pre-implanted human blastomeres using Next Generation Sequencing. With the use of NGS technique, these data represent one of

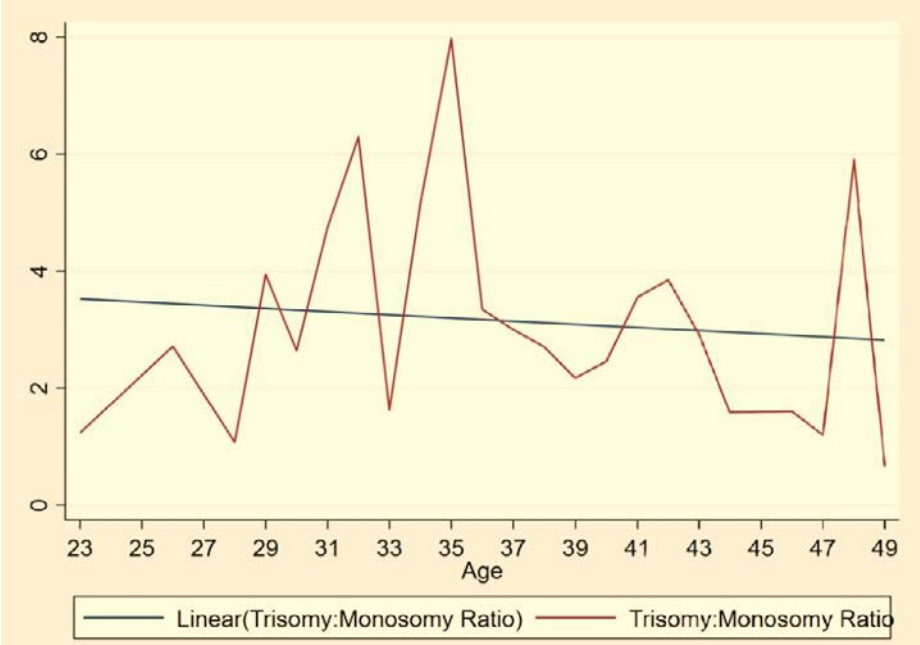

Fig4. Mean trisomy: monosomy ratio within the total blastomere sample according to maternal age.

the limited number of reports using this technology in the State of Kuwait. NGS technique has been standard worldwide, and this is attributed to its high detection rate (98%) of chromosomal aberrations (7). Despite its advantages, higher chances of reporting variants of unknown significance have also been well recognised (3). Overall, 572 (70.8%) of the blastomeres in our studied sample demonstrated an abnormal chromosome complement and hence deemed as aneuploid regardless of maternal age or complexity of the aberration present. We found this result to be in keeping with previous literature and consensus with established estimates of aneuploidy in human IVF embryos documented to be within 50-80-% (8).

This study was also able to explore the relationship between aneuploidy and maternal age in the general IVF population in Kuwait. It has been well documented that a high incidence of chromosomal aneuploidy occurs with maternal ages 35 years and older (7). This association has been known to complicate pregnancies, with chromosomal abnormalities (majority of which were aneuploidy of the embryo) being identified in approximately 50% of first trimester miscarriages (9). Evaluation of the presence of such association has been partially reflected by in our study given the highest percentage of aneuploidy was within blastomeres from maternal ages 40 years and above, making about 72.6% of embryos from this demographic. Overall evaluation of aneuploidy from previous research have not only established an increase in its frequency with maternal age but also demonstrated an increase in

proportion of embryos with more than one aneuploid chromosome (4). Our study has shown no significant difference regarding the complexity of chromosomal aberration within the blastomeres and age of female partner across all age groups. This could partly be due to the limited population sample obtained in the period our study covered, and therefore no such relationship could be inferred. Moreover, when evaluating the trisomy to monosomy ratio of the blastomeres, it was clear that group 1 (youngest maternal age) have demonstrated the lowest trisomy: monosomy ratio (1.235). This finding was in accordance with previous literatures that associated higher ratios with increased maternal ages (4).

Our study was primarily based on the examination of day 3 embryonic division blastomeres from parents with no known chromosomal translocations. Many techniques, including NGS have been employed for PGD at blastocyst stage, which is equivalent to an embryo at day 5 embryonic division, however, only a limited number of studies have reported outcomes on day 3 embryonic division blastomeres (2). We found that equal number of embryos are available at day 3 embryonic division and day 5 embryonic division, and biopsies (in both days) do not adversely affect embryo viability, chromosome configuration and ultrastructure (10, 11). Nevertheless, biopsy at day 3 embryonic division may yet cause an inaccurate assessment of the potential development of a euploid embryo because of the high mosaicism at this stage of development (12). Other technical limiting factors that may play a role in the outcomes and must be acknowledged, are included; timing of embryo biopsy, vitrification, and transfer during IVF procedure.

The limitation of our study was the size of the population. Therefore, larger cohort studies of aneuploidy for further evaluation, increase generalisability, and improve patient counsellings are needed.

Data sharing and availability statement

The data that supports the findings in this study are available from the corresponding author upon reasonable request.

Acknowledgment

No funding resources to declare.

Conflict of interest:

The authors have no conflict of interest with respect to the research, authorship, and publication of this article.

Author contributions:

Dr Abdullah A Albahar designed the work and study. Rana AS Sarmiti and Megdeline G Martin have acquired and collected the data. Dr Abdullah A Albahar have analysed and interpreted the data. Dr Abdullah A Albahar and Megdeline G Martin drafted this article. All authors revised the final manuscript and agreed to be accountable for all aspects of the work. Final approval of the version to be published by Dr Abdullah A Albahar Megdeline G Martin· Eman KH Bahar and Rana AS Sarmiti.

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