**Are all Human Embryos Mosaic?**

**Lauren Kelly, Darren K Griffin**

School of Biosciences, University of Kent

**Abstract**

Preimplantation genetic testing for aneuploidy (PGT-A) is performed to determine whether an embryo has a normal or abnormal chromosomal complement. Diagnoses tend to be reported as “euploid, aneuploid or mosaic” however this primitive classification system refers only to approximately five cells removed from the trophectoderm of a ~200 cell blastocyst. Thus, the mosaicism status of the rest of the embryo remains under-reported and figures that quote the % of biopsies in which mosaicism is detected should not be mistaken

 diagnosis to be made. This well-established technique was first conducted by fluorescent *in situ* hybridisation (FISH).

FISH was successful in providing the cytogenic information of embryonic cells, however, due to the technology’s inability to test for all 24 chromosomes it is no longer used for PGT- A, and instead next generation sequencing (NGS) is the preferred methodology. Unlike FISH, NGS has the ability to analyse all 24 chromosomes and has a greater sensitivity compared to other techniques. Despite the advancements in the technology, controversies still remain surrounding the incidence of aneuploidy and mosaicism of human preimplantation embryos. Conflicting results amongst published literature using FISH or NGS for PGT-A add to the difficulty in determining the true rate of aneuploidy and mosaicism. Furthermore, the presence of a self-correcting mechanism indicates that all human preimplantation embryos contain euploid and aneuploid cells at some point and are therefore mosaic. Through the analysis of published literature and data provided by Cooper Genomics, this novel study demonstrates that reported rates of aneuploidy and mosaicism vary considerably. In addition, the findings show that different approaches of FISH fixation influence the rate in which aneuploidy misdiagnosis occurs. Finally, this study confirms that the chromosomal status of an embryo is dependent on the location of the embryo biopsy, raising the issue that an embryo biopsy is not necessarily a true reflection of the chromosomal status of the whole embryo.

**Introduction**

Human preimplantation embryos undergo genetic testing for aneuploidy (PGT-A) to identify whether or not they carry a normal chromosome complement. They are therefore, as far as can be ascertained, classified euploid if a biopsy sample indicates no chromosome abnormality. Conversely, if they have an abnormal number of chromosomes identified in the biopsy they should be classifying them as aneuploid (Homer, 2019). Aneuploidy can be detected in up to 50% of *in-vitro* fertilization (IVF) human embryos (Shahbazi et al., 2020), and usually results in failed implantation or pregnancy loss (Findikli, 2006). Aneuploidy may arise from chromosome mis-segregation in mitosis and this typically results in mosaicism. Similarly, a meiotic error can cause aneuploidy of every cell

but more often it occurs during meiosis after fertilization due to chromosome misallocation (Compton, 2011; Lee and Kiessling, 2017).

Using PGT-A to identify euploid embryos can improve IVF clinical outcomes, although this is not always the case and some euploid embryos fail to progress to delivery (Lledo et al., 2017). Mosaicism has been offered as an explanation for the failure of euploid embryos after transfer.

A mosaic preimplantation embryo consists of two or more cell lines, each possessing a different chromosome complement (Munne and Wells, 2017), figure 1. However, the criteria defining a mosaic embryo appears to differ amongst research publications. And although a relatively common finding, the prevalence of mosaicism in preimplantation embryos is undefined, with rates of mosaicism varying amongst research studies and IVF laboratories (Kahraman et al., 2020; Wu et al., 2021). Like aneuploid embryos, failed implantation and high miscarriage rates are reported for preimplantation embryos diagnosed by PGT-A as mosaic (Munne, 2018).



**Figure 1.** The development of a mosaic embryo through different stages. Aneuploid cells are in purple and euploid green. The figure demonstrates the effect a defective mitotic event can have on the cellular composition of an embryo (purple represents aneuploid cells with primary karyotype, red and blue are aneuploid cells with secondary karyotype, green represents euploid cells with primary karyotype, and grey represents cell fragments that contain lagging chromosomes). Figure taken from Poli and Capalbo (2020, p. 112).

The diagnosis of mosaicism can be achieved through the biopsy and analysis of two cells from an embryo (Wilton et al., 2009). However, this method does not guarantee the detection of mosaicism in the other embryonic cells. More often, a diagnosis is achieved through analysing embryonic cells already classified as aneuploid after a single-cell biopsy and PGT- A (Wilton et al., 2009). Fluorescence *in situ* hybridisation (FISH) was first used for PGT in the 1990s for the detection of female, male and Turner Syndrome embryos (Parikh et al., 2018). Following this, the technique was used by many for the detection of translocations and aneuploidy in embryos (Parikh et al., 2018). Further developments in the technology led to an improved method and the ability to test 5-12 chromosome pairs using different probe mixtures (Parikh et al., 2018). Alongside the limitations of the technology shown in table 1, varied mosaicism levels have been reported when using FISH, ranging from 20% to more than 90% when analysing blastocysts across different clinics (Wu et al., 2021). There is also debate as to whether the different fixation methods contribute to the error rate seen, with publications suggesting that the methanol: acetic acid method produces less FISH errors compared to the Tween 20 method (Velila et al., 2002). Due to the development of comprehensive molecular approaches for the testing of aneuploidy for all chromosomes, FISH is no longer the recommended technique for PGT-A (Coonen et al., 2020).

Since its demonstrated success in 2013, next generation sequencing (NGS) has become a popular method for PGT-A (Parikh et al., 2018). NGS is a sequencing-based technology capable of analysing all 24 chromosomes, and presents with many advantages, as described in table 1 (Fragouli, 2018; Brunet et al., 2018). Its enhanced sensitivity increases its capability in detecting challenging abnormalities, including mosaicism (Fragouli, 2018).

**Table 1. Advantages and disadvantages of the FISH and NGS methodologies when used for PGT-A.**

|  |  |  |
| --- | --- | --- |
|  | **Advantages** | **Disadvantages** |
| **FISH** | * Provides information on the cytogenic status of the embryonic cells
* Can be performed quickly on single cells at interphase
 | * Unable to detect aneuploidies for all chromosomes
* Poor ability in predicting aneuploidy in blastocysts that are morphologically normal
 |
| **NGS** | * Capable of analysing all 24 chromosomes
* Able to generate large quantities of DNA sequence information
* Cost effective
* Greater sensitivity compared to other approaches (can detect mosaicism levels as low as 20%)
* Results produced in 3-7 hours
 | * Requires an understanding by clinicians to use the technology
* High variance in mosaicism rates across IVF laboratories
 |

(Munne and Wells, 2017; Northop et al, 2010; Brezina et al., 2016; Chen et al., 2020; Fragouli, 2018; Brunet et al., 2018; Lai et al., 2017; and Wu et al. 2021).

The phenomenon of mosaicism means that an embryo may be classed as aneuploid by PGT- A of a trophectoderm (TE) biopsy, but euploid when conducting analysis on cells obtained from the inner cell mass (ICM) (Victor et al., 2018), figure 2. This has raised concerns amongst the scientific community surrounding the discarding of blastocysts diagnosed as aneuploid by a TE biopsy as the ICM of those embryos may be euploid (Victor et al., 2018). Further to this, research is emerging regarding healthy live births following the transfer of mosaic blastocysts (Greco et al., 2015; Kahraman et al., 2020; Lee et al., 2020) which has great potential in improving IVF success.

PGT-A accuracy

**Figure 2.** Types of mosaicism depending on the location of the aneuploid and euploid cells (TE or ICM), and the location of the biopsy. Aneuploid cells are red, and euploid cells blue. Figure taken from Rubio et al., 2020 and adapted.

The purpose of this study is to investigate two methods of mosaicism and aneuploidy detection, FISH and NGS. This novel study will examine the true rate of mosaicism, defined as the number of mosaic embryos divided by the total number of embryos assayed, as well as examine rates of aneuploidy and euploidy amongst human preimplantation embryos.

Analysis into already published studies and novel data provided by Cooper Genomics will be conducted to determine whether all human preimplantation embryos are aneuploid and mosaic, as predicted by the hypothesis of this study.

Materials and Methods

Inclusion and Exclusion Criteria.

Studies included in the research used either FISH or NGS in the detection of aneuploidy, euploidy and mosaicism of human preimplantation embryos, and contained 50 or more embryos in the sample. The testing occurred on day 3 or day 5 of embryo development, with some studies completing follow-up testing. Publications investigating concordance between the TE and ICM of an embryo were also included in the research. Studies using FISH were only considered if they looked at 5 or more chromosomes.

Research publications were excluded if the sample size was less than 50 embryos. Publications that used array CGH, SNP methods and non-invasive testing for aneuploidy were also excluded. Other exclusion criteria included redundant studies and publications that lacked important interpretable data.

Data was also provided by Cooper Genomics. This data analysed rates of aneuploidy, euploidy and mosaicism of human preimplantation embryos using NGS, and was analysed alongside the data collected from published studies.

Literature Search.

Searches were conducted in Google Scholar, PubMed and using the Google search toolbar, and used appropriate phrases and keywords. Keywords included ‘aneuploid’ and ‘mosaic’. The following phrases and abbreviations were also used; ‘preimplantation genetic testing for aneuploidy’ (PGT-A), ‘fluorescence in situ hybridization’ (FISH), ‘next generation

sequencing’ (NGS), ‘inner cell mass’ (ICM), and ‘trophectoderm’ (TE). No date restrictions were implemented when conducting searches.

Data Extraction Pooling.

Relevant data from the publications was extracted directly, with reported values stated as they were by authors in the original publication. In some instances, values were calculated, basing calculations on the data in the original publications.

Statistical Analysis.

All analysis was performed using Excel and SPSS, with all calculations documented to 3 significant figures (3.s.f). Mean aneuploidy and mosaicism rates of the published data were calculated for both the FISH and NGS studies. Rates of aneuploidy misdiagnosis were compared by method of fixation by conducting an independent sample T-test. A significance level was set at p<0.05, and 95% confidence intervals were calculated.

Excel was also used to model the possible diagnosis of a ‘virtual’ 5 cell embryo biopsy. Upon entering a random iteration into the model, a resulting biopsy diagnosis of either euploid, mosaic or aneuploid was predicted.

**Results**

Study Characteristics and Literature Search.

Searches led to the identification of 42 publications that fit the desired criteria. Studies included were published between 1998 and 2021. The publications were categorised into 2 groups depending on the method used for PGT-A. The main characteristics of the studies using FISH are displayed in table 2, and the characteristics of the publications using NGS presented in table 3.

**Table 2. Overview of publications using FISH for PGT-A that fit the inclusion criteria.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***Reference*** | ***Number of chromosomes analysed*** | ***Number of embryos in sample*** | ***Method*** | ***Euploidy Rate (%)*** | ***Aneuploidy Rate (%)*** | ***Mosaicism Rate (%)*** | ***No result (%)*** | ***Re- analysis*** |
| *Magli et al., 1998* | 6 | 412 | n/a | 38.8 | 56.8 | n/a | 4.40 | No |
| *Munne et al., 1998* | 6 | 67 | Methanol: acetic acid, 3:1 | 29.9 | 23.9 | 46.2 | n/a | Yes |
| *Gianaroli et al., 1999* | 9 | 148 | Methanol: acetic acid, 3:1 | 5.4 | 71.0 | 23.6 | n/a | Yes |
| *Magli et al., 2000* | 6 | 143 | Methanol: aceticacid, 3:1 | 49.0 | 51.0 | n/a | n/a | No |
| *Sandalinas et al., 2001* | 9 | 215 | Methanol: acetic acid, 3:1 | 14.9 | 47.0 | 38.1 | n/a | Yes |
| *Bienlanska et al., 2002* | 9 | 216 | Tween 20 | 29.7 | 22.2 | 48.1 | n/a | Yes |
| *Ziebe et al., 2003* | 7 | 103 | Tween 20 | 31.1 | 8.70 | 55.3 | 4.90 | No |
| *Abdelhadi et al., 2003* | 13 | 426 | Methanol: aceticacid, 3:1 | 23.3 | 44.1 | 32.6 | n/a | Yes |
| *Munne et al., 2003* | 9 | 1071 | Methanol: acetic acid, 3:1 | 29.6 | 45.4 | 25.0 | n/a | Yes |
| *Jones et al., 2004* | 7 | 411 | n/a | 34.8 | 62.5 | n/a | 2.70 | No |
| *Ye et al., 2004* | 5 | 54 | Tween 20 | 44.4 | 50.0 | n/a | 5.60 | No |
| *Baart et al., 2005* | 10 | 196 | Tween 20 | 35.7 | 33.2 | 31.1 | n/a | Yes |
| *Li et al., 2005* | 5 | 660 | Tween 20 | 55.6 | 42.6 | n/a | 1.80 | Yes |
| *Cooper et al., 2006* | 8 | 51 | Methanol: acetic acid, 3:1 | 31.4 | 33.3 | 35.3 | n/a | Yes |
| *Mantzouratou et al., 2007* | 6 | 354 | Tween 20 | 0.28 | 5.37 | 94.9 | 0.00 | Yes |
| *DeUgarte et al., 2008* | 5 | 241 | Tween 20 | 24.5 | 68.0 | 7.50 | n/a | Yes |
| *Hanson et al., 2009* | 7 | 149 | Tween 20 | 4.00 | 65.8 | 30.2 | n/a | Yes |
| *Alegretti et al., 2009* | 9 | 75 | n/a | 30.7 | 69.3 | n/a | n/a | No |
| *Barbash-Hazan et al., 2009* | 8 | 83 | Tween 20 | 0.00 | 79.5 | 18.1 | 2.40 | Yes |
| *Mir et al., 2010* | 9 | 2477 | Methanol: acetic acid, 3:1 | 42.6 | 56.8 | 0.60 | n/a | Yes |
| *Ebrahimian et al., 2020* | 8 | 68 | Methanol: acetic acid, 3:1 | 67.6 | 17.7 | 14.7 | n/a | Yes |

For studies that completed PGT-A re-analysis, rates are reported as determined by the re-analysis. Not all articles reported rates of euploidy, aneuploidy and mosaicism as percentages, and so percentages were calculated in these instances. All percentages were calculated to 3.s.f.

n/a refers to studies not reporting the data.

When analysing those studies that used FISH, the number of embryos in each study ranged from 51 to 2477, table 2. Re-analysis of embryos occurred in 15 of the FISH studies, with the rate in which aneuploidy was misdiagnosed calculated for 13 (appendix 6.1).

**Table 3. Overview of publications using NGS for PGT-A that fit the inclusion criteria.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Reference*** | ***Number of embryos in sample*** | ***Euploidy Rate (%)*** | ***Aneuploidy Rate (%)*** | ***Mosaicism Rate (%)*** | ***No Result (%)*** | ***Re-analysis*** |
| *Ruttanajit et al., 2015* | 399 | 48.9 | 38.1 | 13.0 | n/a | No |
| *Bono et al., 2015* | 145 | 13.8 | 84.8 | 1.38 | n/a | No |
| *Zhang et al. 2016* | 98 | 30.6 | 66.3 | 3.06 | n/a | No |
| *Xu et al., 2017* | 108 | 77.6 | 22.4 | n/a | n/a | No |
| *Viotti et al., 2017* | 50 | 6.00 | 94.0 | n/a | n/a | Yes |
| *Elkhatib et al., 2018* | 88 | 4.8 | 95.2 | n/a | n/a | Yes |
| *Linan et al., 2018* | 118 | 40.7 | 59.3 | n/a | n/a | Yes |
| *Victor et al., 2018* | 100 | 5.00 | 95.0 | n/a | n/a | Yes |
| *Eibes et al., 2019* | 173 | 8.70 | 91.3 | n/a | n/a | No |
| *Lawrenz et al.,2019* | 84 | 52.4 | 47.6 | n/a | n/a | Yes |
| *Dang et al., 2019* | 603 | 57.3 | 42.7 | n/a | n/a | No |
| *Rodrigo et al., 2020* | 111,860 | 46.1 | 53.8 | 0.10 | n/a | Yes |
| *Girardi et al., 2020* | 78 | 32.1 | 21.8 | 46.1 | n/a | Yes |
| *Narvatil et al., 2020* | 89 | 17.8 | 58.0 | 24.2 | n/a | Yes |
| *Ou et al., 2020* | 63 | 3.20 | 85.7 | 11.1 | n/a | Yes |
| *Chuang et al., 2021* | 3,966 | 44.1 | 26.3 | 29.6 | n/a | No |
| *Ye et al., 2021* | 1239 | 20.7 | 73.6 | n/a | 5.70 | No |
| *Alyafee et al., 2021* | 200 | 23.0 | 58.5 | 1.50 | 17.0 | Yes |
| *Wu et al., 2021* | 1719 | 38.8 | 29.2 | 29.2 | 2.80 | Yes |
| *Tong et al. 2021* | 591 | 50.8 | 39.9 | 9.30 | n/a | No |
| *Li et al.,2021* | 3738 | 23.2 | 61.3 | 14.9 | 0.60 | Yes |

For studies that completed PGT-A re-analysis, rates are reported as determined by the re-analysis. Not all articles reported rates of euploidy, aneuploidy and mosaicism as percentages, and so percentages were calculated in these instances. All percentages were calculated to 3.s.f.

n/a refers to studies not reporting the data.

For the NGS studies, the sample of embryos ranged from 50 to 111,860, table 3. Follow up analysis occurred in 12 of the 21 NGS studies.

Reference

Incidence of Aneuploidy.

The reported rates of aneuploidy ranged greatly, regardless of the method used. The rate of aneuploidy in the FISH studies ranged from 5.37% to 79.5%, and in the NGS studies from 21.8% to 95.2%. The mean rate of aneuploidy was slightly higher in studies using NGS compared to FISH; 59.3% (95% CI; 47.9%-70.6%) and 44.9% (95% CI; 35.5%-54.3%)

respectively, figure 3. a)

b)

Reference

**Figure 3. Aneuploidy rate of embryos**. a) Aneuploidy rate expressed as a percentage for embryos that underwent PGT-A by FISH. Individual studies are shown in ascending order by year of publication, with the mean presented at the top. b) Aneuploidy rate expressed as a percentage for embryos that underwent PGT-A by NGS. Individual studies are shown in ascending order by year of publication, with the mean presented at the top. (All percentages and CIs calculated to 3.s.f).

Single Aneuploidies - FISH.

Analysis into the presence of single aneuploidies was conducted in 3 of the 21 publications using FISH, table 4. Of these publications, 2 reported the presence of monosomy 13; Cooper et al., 2006, and Munne et al., 1998. If all chromosomes were tested for aneuploidy in these cases, taking into consideration the 5% error rate of FISH and that a second trisomy could arise, it could be estimated that the chances of this single aneuploidy occurring is slightly less than 3% for the Cooper et al. study, and slightly less than 4% for the Munne et al. study.

When analysing the rate of trisomy, both Munne et al., 1998, and Baart et al., 2005, reported the presence of trisomy 21, stating its occurrence 3 times and once respectively, table 4.

Again, taking into consideration the possible caveats mentioned above, if all chromosomes were tested it can be assumed that the likelihood of a trisomy 21 occurring is slightly less than 12% in the Munne et al study, and slightly less than 2.4% in the Baart et al publication.

**Table 4. Frequency of single aneuploidies as reported in the original publications.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | *Cooper et al., 2006* | *Munne et al., 1998* | *Baart et al., 2005* |
| *Monosomy 13* | 1 | 1 | 0 |
| *Monosomy 15* | 2 | 0 | 0 |
| *Monosomy 16* | 1 | 2 | 0 |
| *Monosomy 18* | 2 | 2 | 0 |
| *Monosomy 21* | 4 | 0 | 1 |
| *Monosomy 22* | 6 | 0 | 1 |
| *Monosomy X* | 1 | 0 | 0 |
| *Trisomy 16* | 0 | 2 | 0 |
| *Trisomy 21* | 0 | 3 | 1 |
| *Trisomy 22* | 0 | 0 | 2 |
| *Trisomy X* | 0 | 2 | 0 |

Frequencies are listed as they were reported in the original publications. This table only presents some of the single aneuploids described in the 3 articles.

Incidence of Mosaicism.

As shown in table 2, reported rates of mosaicism varied greatly in the studies using FISH, with some reporting virtually no mosaicism, 0.6% and others reporting rates as high as 94.9%. The range for the NGS publications was slightly less, ranging from 0.1% to 46.1%, table 3. The average rate of mosaicism was calculated to be higher amongst the FISH studies compared to the NGS studies, 33.4% (95% CI; 20.9-46.0) and 15.3% (95% CI; 6.20-24.4)

respectively, figure 4.

a)

Mean

Ebrahimian et al., 2020

Mir et al., 2010 Hanson et al., 2009 Barbash-Hazan et al., 2009 DeUgarte et al., 2008 Mantzouratou et al., 2007 Cooper et al., 2006

Baart et al., 2005

Ziebe et al., 2003

Munne et al., 2003 Abdelhadi et al., 2003 Bienlanska et al., 2002 Sandalinas et al., 2001 Gianaroli et al., 1999

Munne et al., 1998

33.4 (95% CI; 20.9-46.0)

0 10 20 30 40 50 60 70 80 90 100

Mosaicism Rate (%)

Reference

b)

Mean Li et al., 2021 Tong et al. 2021 Wu et al., 2021 Alyafee et al., 2021 Chuang et al., 2021 Ou et al., 2020

Narvatil et al., 2020 Girardi et al., 2020 Rodrigo et al., 2020 Zhang et al. 2016 Bono et al., 2015

Ruttanajit et al., 2015

15.3 (95% CI 6.20-24.2)

0 10 20 30 40 50 60 70

80

90

100

Mosaicism Rate (%)

Reference

**Figure 4. Mosaicism rate of embryos**. a) Mosaicism rate expressed as a percentage for embryos that underwent PGT-A by FISH. Individual studies are shown in ascending order by year of publication, with the mean presented at the top. b) Mosaicism rate expressed as a percentage for embryos that underwent PGT-A by NGS. Individual studies are shown in ascending order by year of publication, with the mean presented at the top. (Only studies analysing mosaicism rates are included in these figures, all percentages and CIs calculated to 3.s.f).

Methanol: acetic acid, 3:1, Vs. Tween 20 - FISH.

The percentage of embryos originally diagnosed as aneuploid by FISH and re-diagnosed as euploid on follow up analysis was calculated (appendix 6.1). The data was then categorised depending on fixation method to determine if the method of fixation influenced the results, figure 5. False diagnosis was more common in studies using the method Tween 20 compared to methanol: acetic acid, with the difference found to be statistically significant.

50

\*

45

25.4 (95% Cl; 7.36-43.4)

40

35

30

25

20

12.9 (95% CI; 8.37-17.5)

15

10

5

0

Methanol:acetic acid, 3:1

Tween 20

Fixation Method

Error Rate (%)

**Figure 5. Mean error rate of aneuploidy misdiagnosis categorised by FISH fixation method.** The mean error rate for studies using methanol: acetic acid, 3:1: (n=8) and Tween 20 (n=5); p=0.048, independent sample T-test. Error bars represent 95% CI. (Means and CIs calculated to 3.s.f.).

Re-analysis of PGT-A – NGS.

Of the 21 NGS studies, 6 investigated the concordance between the TE and ICM when diagnosed as aneuploid, euploid or mosaic, table 5. Although it appears that the Lawrenz et al., 2019, study shows full concordance, this is not the case as upon further analysis it is apparent that those embryos diagnosed as euploid and aneuploid differ, with different chromosomal rearrangements present.

**Table 5. Comparison of TE and ICM diagnosis as reported by studies using NGS.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***Reference*** | ***Number of embryos in sample*** | ***TE******Euploidy Rate (%)*** | ***ICM******Euploidy Rate (%)*** | ***TE******Aneuploidy Rate (%)*** | ***ICM******Aneuploidy Rate (%)*** | ***TE******Mosaicism Rate (%)*** | ***ICM******Mosaicism Rate (%)*** |
| *Narvatil et al., 2020* | 89 | 17.8 | 27.2 | 58.0 | 55.3 | 24.2 | 17.5 |
| *Ou et al., 2020\*\** | 63 | n/a | 2.94 | 100 | 85.7 | n/a | 11.1 |
| *Lawrenz et al.,2019* | 84 | 52.4 | 52.4 | 47.6 | 47.6 | n/a | n/a |
| *Victor et al., 2018\*\** | 100 | n/a | 7.00 | 100 | 93.0 | n/a | n/a |
| *Viotti et al., 2017\*\** | 50 | n/a | 6.00 | 100 | 94.0 | n/a | n/a |
| *Elkhatib et al., 2018* | 88 | 3.4 | 4.80 | 96.6 | 95.2 | n/a | n/a |

\*\* Aneuploidy originally reported as 100%, but changes upon re-analysis of ICM and TE. n/a refers to studies not reporting the data.

Modelling Aneuploidy and Mosaicism.

Through statistical modelling it can be suggested that when conducting PGT-A on an embryo biopsy of 5 cells the resulting diagnosis is most likely to be mosaic. Figure 6 displays the rate at which euploidy, aneuploidy and mosaicism may occur in human embryo biopsies; 36.7%, 0%, and 63.3% respectively.

**Figure 6. The predicted frequency of euploid, aneuploid and mosaic embryo biopsies using a virtual model. 5** represents a euploid diagnosis (n=110); **4+1** (n=116), **3+2** (n=57), **2+3** (n=15), and **1+4** (n=2) represent a mosaic diagnosis; and **5** represents an aneuploid diagnosis (n=0). Green numbers represent a euploid cell and red numbers an aneuploid cell. The model assumes an embryo biopsy of 5 cells.

140

120

100

80

60

40

20

0

**5**

**4+1**

**3+2**

**2+3**

**1+4**

**5**

Possible Diagnosis

PGTai 2.0 (NGS) Data.

When investigating the diagnosis of PGT-A using NGS and artificial intelligence (AI), a euploid diagnosis occurred more frequently than a mosaic or aneuploidy diagnosis, table 6.

**Table 6. PGTai 2.0 (NGS) data as reported by Cooper Genomics.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | *Euploid* | *Mosaic* | *Aneuploid* | *Total* |
| *<35* | 15287 | 4747 | 7810 | 27844 |
| 55% | 17% | 28% |  |
| *35-37* | 11918 | 3765 | 8181 | 23864 |
| 50% | 16% | 34% |  |
| *38-40* | 9471 | 3376 | 10809 | 23656 |
| 40% | 14% | 46% |  |
| *41-42* | 3156 | 1465 | 7339 | 11960 |
| 26% | 12% | 61% |  |
| *>42* | 1381 | 738 | 6983 | 9102 |
| 15% | 8% | 77% |  |
| *Egg Donor* | 5246 | 1414 | 2230 | 8890 |
| 59% | 16% | 25% |  |
| *Total* | 46459 | 15505 | 43352 | 105316 |
| 44% | 15% | 41% |  |

105316 embryo biopsies equate to around 22,000 cycles. All egg donors are assumed to be under 35 years old.

Discussion

It is evident that aneuploidy and mosaicism rates of human preimplantation embryos vary greatly amongst published literature, figures 3 and 4. In addition to this, from the analysis of such published literature, it can be concluded that combined rates of aneuploidy and mosaicism are greater than euploidy rates, with this being the case for all but 3 NGS and 2 FISH studies. This finding therefore suggests that human preimplantation embryos are more likely to be either aneuploid or mosaic compared to euploid.

The results documented in the literature are somewhat reflected in the data provided by Cooper Genomics, as the combined total of aneuploidy and mosaicism rates are greater than euploidy rates, table 6. Furthermore, when comparing to published NGS studies, the total mosaicism rate of the Cooper data is very similar to the mean mosaicism rate of the NGS studies, 15% and 15.3% respectively. However, the aneuploidy rates are quite different, with the mean rate at which aneuploidy is reported for the NGS studies at 59.3% compared to a reported rate of 41% when using PGTai 2.0. Cooper Genomics uses AI technology when conducting PGT-A, and so it may be that this data is more accurate when reporting aneuploidy and mosaicism rates.

Mosaic and aneuploid human embryos are not uncommon (Kahraman et al., 2020; Shahbazi et al., 2020). Cell division mistakes occur frequently during the process of human embryogenesis and are thought to be a causative factor in embryonic mosaicism and aneuploidy (Orvieto et al., 2020), thus offering an explanation as to why aneuploidy and mosaicism rates are relatively high. Nonetheless, when solely looking a mosaicism, it is possible that such rates are higher than what is reported. The virtual embryo biopsy model presents a mosaicism rate of 63.3%, figure 6, a value that is higher than both the reported literature and the data from Cooper Genomics. On the proviso that this model is an accurate representation of an embryo biopsy diagnosis, it suggests that mosaicism rates are under- reported.

A possible explanation for the different mosaicism rates seen in the virtual model and amongst the reported data may be directly correlated to the time at which the embryo biopsy took place. As described by Esfandiari et al., 2016, mosaicism rates are far less reported at the blastocyst stage compared to cleavage stage embryos. The published literature presented in this study reflects PGT-A results conducted on embryos at both the cleavage and blastocyst stage, and so if only cleavage stage results were reported it may be that the mosaicism rates are higher.

When an embryo presents with aneuploidy cells, it is suggested that a self-correcting mechanism occurs. This is thought to happen between the cleavage and blastocyst stage of embryo development, resulting in higher rates of euploidy at the blastocyst stage (Barbash- Hazan et al., 2009). Theories behind how such a mechanism operates suggest there is an increase in aneuploidy cell death, or a decrease in cell division rate (Santos et al., 2010), resulting in a euploid blastocyst. The presence of this mechanism in embryos therefore offers an explanation as to why euploidy, aneuploidy and mosaicism rates differ amongst the literature. The mechanism also suggests that at some point in development, an embryo diagnosed as euploid was once aneuploid.

As presented in this study, an embryo diagnosis can differ depending on the location of the biopsy, table 5. Published literature indicates a higher rate of aneuploidy in the TE of an embryo, and higher euploidy rates in the ICM (Narvatil et al., 2020; Ou et al., 2020; Lawrenz et al., 2019; Victor et al., 2018; Viotti et al., 2017; Elkhatib et al., 2018). This difference indicates the presence of mosaicism, and so embryos should be given a mosaic diagnosis.

A blastocyst stage biopsy is thought to be more representative of an embryo’s chromosomal status compared to a cleavage stage biopsy. However, as described by Chuang et al., 2018, it is also possible that the chromosomal status of cells located in the TE can differ. Such findings lead us to question the reliability of a TE biopsy. The possibility that the chromosomal status of cells found in the ICM of an embryo can differ rather dramatically from those found in the TE, and that the chromosomal status of TE cells can differ also, suggests that a TE biopsy is not necessarily an accurate representation of an embryo. This idea is further corroborated by the output of the virtual embryo model, figure 6. The diagnosis of mosaicism differs amongst the biopsies, with some predicted to have 1 euploid cell and 4 aneuploid, and others 2 euploid cells and 3 aneuploid, and so on. This demonstrates the different rates at which mosaicism can occur within a biopsy. Because of these findings, a better way forward may be to categorise an embryo following PGT-A as a euploid embryo biopsy, rather than a euploid embryo.

The presence of different cell lines amongst the ICM and TE indicate an embryo to be mosaic. However, it is worth noting that such a term is not accepted by all in the scientific community. Some deem the term mosaic to be ‘inaccurate’ and ‘misleading’, on the basis that it is of poor clinical predictive value and so believe it should be abandoned (Paulson and Treff, 2020). Despite these claims, the findings presented in this study show that mosaicism does exist amongst embryos, and as a result these mosaic embryos could also be classed as aneuploid due to their abnormalities. And so, rather than dismissing the term mosaicism, I think it is important to acknowledge it, as well as the case that these mosaic embryos are also aneuploid.

This study analyses the use of both FISH and NGS in PGT-A. Aneuploidy rates appear to be higher amongst the NGS studies, and mosaicism rates higher amongst studies using FISH. This finding may be different to what is expected on the basis that the sample of embryos in the NGS studies are more likely to be blastocyst stage embryos, compared to a higher proportion of cleavage stage embryos used in FISH studies due to the advancements of embryo culture medium. As mentioned previously, higher rates of aneuploidy are expected amongst cleavage stage embryos due to the self-correcting mechanism that occurs between day 3 and 5 of embryo development (Barbash-Hazan et al., 2009). Nevertheless, NGS is a more accurate way of detecting embryo aneuploidy, as described in table 1, and so the difference in embryo diagnosis may be a result of the inaccuracy of FISH.

Upon analysis of studies using FISH for PGT-A, it can be concluded that using Tween 20 for slide fixation results in an increased rate of misdiagnosis, figure 5. However, due to the small sample sizes involved in this analysis, this finding is not concrete. Nonetheless, a study by Velilla et al., 2002, corroborates this finding, with their research suggesting Tween 20 results in poor nuclear quality, a number of signal overlaps, and a higher rate of errors. Due to this, consideration should be taken when examining PGT-A results as determined by FISH using Tween 20. Reliability of PGT-A results has since been enhanced through improvements in technology and the use of AI as it results in non-subjective analysis.

Despite the findings of this study, the research does not present without its limitations. Some of the publications in this study performed PGT-A on embryos already classified as poor quality, and so higher aneuploidy rates might be expected. Furthermore, this study does not delve deep into the differences between day 3 and day 5 biopsy results when analysing the published literature, something that ought to be considered if conducting this research again on a larger scale. It would also be worthwhile reviewing the virtual embryo model. The model fails to account for cell clumping found in the TE of an embryo when aneuploidy is present, and so results may differ if this was considered.

Nonetheless, the findings of this study have the potential to impact how we view mosaicism and aneuploidy. And although not investigated in this research, other publications have demonstrated the potential of mosaic embryos for embryo transfer, resulting in live births (Lee et al., 2020). Because of this, it is important that embryos do not get discarded on the basis of a mosaic diagnosis and are instead considered for embryo transfer, especially if we are already transferring euploid embryos that were potentially once aneuploid and thus mosaic.

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Aneuploidy misdiagnosis rates from FISH studies that completed PGT-A reanalysis.

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| ***Reference*** | ***Aneuploidy Misdiagnosis Rate******(%)*** |
| *Munne et al., 1998* | 14.9 |
| *Gianaroli et al., 1999* | 5.41 |
| *Sandalinas et al., 2001* | 7.08 |
| *Abdelhadi et al., 2003* | 10.6 |
| *Munne et al., 2003* | 12.4 |
| *Baart et al., 2005* | 33 |
| *Li et al., 2005* | 40 |
| *Cooper et al., 2006* | 22.2 |
| *DeUgarte et al., 2008* | 17.17 |
| *Hanson et al., 2009* | 4.14 |
| *Barbash-Hazan et al., 2009* | 32.5 |
| *Mir et al., 2010* | 17.8 |
| *Ebrahimian et al., 2020* | 13.2 |

Percentages are either presented as reported by the original publication or calculated using the data reported by the studies. All percentages are written to 3.s.f.