RBMO

REVIEW

Controversies in ART: can the IVF laboratory influence preimplantation embryo aneuploidy?

BIOGRAPHY

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KEY MESSAGE

As reported, rates of embryo aneuploidy vary between IVF centres. Attention has focused on possible stressors in the IVF laboratory that may influence chromosome separation and segregation. Differences in blastocyst mosaicism rates could indicate laboratory causes of chromosomal errors. Possible mitotic stressors include pH, osmolality, temperature, oxygen tension and culture media.

ABSTRACT

Published reports have indicated that rates of preimplantation embryo aneuploidy in a control donor population may vary between IVF centres. This suggests that location-specific conditions, in the clinic, IVF or genetics laboratory, may be influencing the chromosome dynamics or diagnosis. More recent reports suggest that rates of embryo mosaicism, representing mitotic errors, may vary between IVF centres. This would suggest perhaps a laboratory-controlled variable is influencing mitotic cell division during preimplantation embryo development. Various IVF laboratory-controlled factors may be impacting chromosome separation and segregation. Variables including type of culture media, pH, temperature, osmolality and oxygen concentration could all be possible factors influencing embryo aneuploidy. Furthermore, laboratory techniques, method of insemination, laser use or handling of biopsied cells may also influence genetic results. These IVF laboratory variables will be reviewed and literature suggesting a possible link to mitotic aneuploidy/mosaicism discussed.

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KEYWORDS

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INTRODUCTION

Variation in techniques and protocols between IVF centres is well accepted and may manifest as differences in successful outcomes. The cause of these outcome disparities may lie in the patient population or clinical approaches employed to treat the infertility. However, differences in IVF outcomes between centres may also be due to conditions within the embryology laboratory and resulting embryo quality. Studies using egg donors as a control population have demonstrated that rates of resulting embryo euploidy differ between IVF centres by as much as 40% (*Munne et al., 2017*). Whether these differences were from a meiotic or mitotic origin is unknown. If meiotic in nature, all cells in the resulting embryo are impacted, and focus may be on the clinical practices and stimulation protocols and impact on the oocyte. If mitotic in nature, then focus may be on the IVF laboratory and culture conditions during embryo development. The resulting embryos may not necessarily be entirely aneuploid and the resulting mosaicism would indicate a possible mitotic origin. Indeed, prior commentary has discussed the possible link between the IVF laboratory culture conditions and embryo mosaicism (*Munne and Alikani, 2011*), although data assessed were sparse and largely derived from early culture system, cleavage-stage embryos and fluorescence in-situ hybridization (FISH) analysis (*Munne et al., 1997*) and subject to the limitations therein.

Fortunately, new genetic analysis platforms now have the sensitivity to more reliably detect mosaicism from biopsied blastocyst samples. Several preliminary studies indicate that rates of embryo mosaicism differ between IVF centres, with some reporting differences as high as 30% (*Sachdev et al., 2016; Wells et al., 2016*). This indicates significant differences between centres and suggests that conditions within the modern IVF laboratory could be influencing mitotic division errors during extended embryo culture.

This review attempts to provide a broad coverage of peer-reviewed data and published abstracts, focusing on human models, but also presenting supporting animal data, both prospective and retrospective in nature, to add to the growing body of literature suggesting a link between conditions used in the clinical IVF laboratory and resulting embryo mosaicism.

CULTURE SYSTEM STRESS

It is well accepted that minimizing harmful cellular stress within the IVF laboratory is required to optimize embryo development and assisted reproductive outcomes. This is especially important considering the sensitive nature of the gametes and embryos. Potential cellular stressors in the IVF laboratory include various mechanical and chemical factors (FIGURE 1). Importantly, some of these stressors may compromise the machinery responsible for proper separation and segregation of chromosomes during mitosis, which may lead to mitotic errors and could manifest as mosaicism in the preimplantation embryo.

However, isolating possible causative environmental stressors in the IVF laboratory and demonstrating a direct impact on mitotic errors is a difficult endeavour. The culturing of preimplantation embryos *in vitro* entails implementation of a culture system, with over 200 variables identified and various aspects being intertwined and interdependent (*Pool et al., 2012*). Some of the key environmental stressors experienced during embryo culture and their possible link to inducing mitotic errors are discussed.

Culture media

One of the most widely studied components of the embryo culture system is culture media. Commercial production of media formulated specifically for preimplantation embryos now exists and provides a level of consistency for laboratories that was previously lacking. A variety of media are available, both single-step and sequential approaches, which support high rates of embryo development and clinical outcomes. While a comprehensive comparison of all media is practically impossible, a systematic review of comparative studies failed to identify a superior medium or approach (*Mantikou et al., 2013*). Notably, this analysis was limited in nature and also did not examine possible impact on aneuploidy or mosaicism.

Culture medium supplies metabolic substrates, antioxidants and other factors that can directly impact embryo cellular processes. Medium components can also degrade and create harmful stressors to the cells. As embryos are directly exposed to these media for

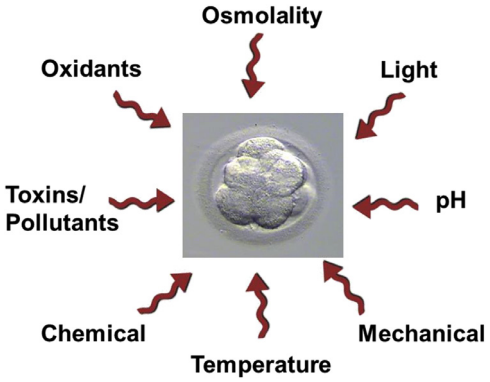

FIGURE 1 Common stressors within the culture system that impact embryo development *in vitro* and may potentially impact machinery responsible for proper mitotic cell division and separation/segregation of chromosomes. While each stressor alone may not cause a significant increase in aneuploidy, subtle stress from multiple sources may yield a cumulative effect that could explain wide variations in embryo aneuploidy between laboratories reported in the literature.

TABLE 1 SUMMARY OF STUDIES EXAMINING IMPACT OF CULTURE MEDIA ON EMBRYO ANEUPLOIDY AND/OR MOSAICISM

Media compared	Result summary	Comments/limitations	Reference
Single versus sequential	Increased mosaicism in single-step media (~4% increase) compared with sequential	Retrospective analysis of 27 clinics with numerous variables involved	*Fragouli et al., 2017*
Single versus single	Sibling embryo splits – differences in mitotic aneuploidy between media	Different proteins used (complex versus HSA) and different media pH (7.14 vs 7.27)	*Hickman et al., 2016*
Sequential versus single	No difference in mosaicism (<2% in each media); lower aneuploidy in single-step in 40–41-year-old patient age group	Retrospective, performed over different time periods, no sibling embryo splits	*Behr et al., 2018*
Single versus single versus single	No differences in blastocyst aneuploidy	Retrospective, no sibling embryo splits	*Wun et al., 2017*
Single versus single	No differences in overall aneuploidy; less aneuploidy in 'high-risk' group in one media (~30% lower)	Sibling embryo splits, low sample size	*Desai et al., 2017*
Single versus sequential	Significantly less aneuploidy in single-step media (~9% less)	Retrospective, no embryo splits	Vermilyea *et al.*, 2018
Single versus single	Less aneuploidy in a low lactate single-step media (~9% less)	Retrospective, no embryo splits	Vermilyea *et al.*, 2018
Single versus sequential	No differences in aneuploidy	Sibling embryo split, pH well controlled between media	*Werner et al., 2016*
Single versus sequential	No differences in aneuploidy	Retrospective, no sibling embryo splits, different incubators/dishes	*Cimadomo et al., 2018*

extended periods, examination of their impact on embryo quality is potentially useful. Several preliminary studies have attempted to determine whether various culture media may be impacting mitotic aneuploidy (TABLE 1). One compared rates of embryo mosaicism between 27 clinics, examining various laboratory factors (*Fragouli et al., 2017*). Mosaicism rates ranged from 11% to 27% and the type of culture medium was the only variable found to be statistically significant, with differences apparent between three single-step media as well as differences between three sequential media. Interestingly, it was also reported that single-step medium yielded higher rates of mosaicism (17%) compared with sequential medium (13%). Unfortunately, this study was retrospective in nature and did not utilize sibling embryo splits to limit the variables involved (*Fragouli et al., 2017*). Thus, it is difficult to ascertain whether any observations are due to the medium alone, or due to other factors between centres and within the culture system that can influence medium performance.

Another retrospective study examined outcomes from two laboratories over a 2-year period, comparing a sequential media system to a low lactate continuous single step in one laboratory and comparing the same low lactate continuous single-step medium to a different continuous single-step medium in a second laboratory (*Vermilya et al., 2018*). Incubators for all comparisons were kept at 6% CO_2. Authors reported that the low lactate medium yielded significantly more euploid blastocysts in both laboratories. Unfortunately, sibling embryo splits were not used to control for various other factors. Although not studied, and purely speculative in nature, rationale for possible improvement in euploidy rate for the low lactate medium could be due to reduced metabolic stress for the embryos, or perhaps more appropriate internal pH (pH_i), as high lactate can lower pH_i.

Two preliminary studies compared blastocyst aneuploidy rates using sibling embryo splits between two single-step media (*Desai et al., 2017; Wun et al., 2017*). Neither indicated any significant differences in overall aneuploidy rates between media. However, one study did indicate higher rates of aneuploidy in one medium compared with the other in a subset of 'high-risk' patients, although only 16 and 18 embryos were analysed (*Desai et al., 2017*). At least one preliminary study indicated no impact on embryo aneuploidy with renewal versus uninterrupted use of a single-step medium (*Ryu et al., 2018*). Impact on mosaicism was not reported.

A preliminary study compared a sequential medium to a single-step medium using sibling embryo splits and identified significant differences in mitotic errors in resulting blastocysts (*Hickman et al., 2016*). Of note, type of protein differed (complex protein with globulins versus human serum albumin HSA). Additionally, incubator CO_2 was not adjusted, resulting in a different pH for each media (7.27 versus 7.14), with the higher rates of mitotic errors occurring with the lower pH, which was outside the recommended range of most media manufacturers. Thus, the impact observed cannot be assigned to culture medium alone and points to the severe flaw in many retrospective studies that attempt to assign outcomes to a particular culture medium. In this case, outcomes could differ based on the protein, or use of an improper pH (discussed later).

A separate study again retrospectively analysed a sequential medium versus a single-step medium over two separate 5-month periods (*Behr et al., 2018*). CO_2 levels were adjusted accordingly to ensure each medium was within the appropriate pH range. Differences in blastocyst formation rates were noted and there was lower aneuploidy in the single-step group in patients >40 years old. Aneuploidy rates were similar in younger age groups and no differences in mosaicism were noted (<2% in both groups). While the study design was flawed, which limited the drawing of conclusions about the impact of the medium alone, this could suggest some embryo- or patient-specific sensitivity and that a group of patients/embryos may benefit from one medium type over another.

One well-designed prospective randomized study compared a sequential system to a single-step medium and examined impact of the two media systems on embryo development and aneuploidy. Different incubators were used to ensure optimal pH values for each medium. Although rates of blastocyst formation differed between treatments, no differences in aneuploidy

were apparent (*Werner et al., 2016*). Mosaicism was not specifically reported.

If differences are apparent between systems/media, it is unknown whether they may be due to formulation differences and metabolic stress due to availability of specific substrates, build-up of harmful by-products such as ammonia or volatile organic compounds, or due to reduced handling/stress (pH, thermal, shear and others). Importantly, how specific media are used, with special consideration of other impactful culture variables, such as protein, incubator conditions and handling, are important factors in media efficacy and their possible impact on embryo mitotic errors.

pH

The impact of pH on embryo development is well known. The pH of culture medium (pH_e), along with effects of bicarbonate and/or CO_2 levels, can impact embryo development (*Quinn, 2012; Swain 2012, 2015; Swain and Pool, 2009*). Denuded oocytes, early stage embryos and cryopreserved/warmed embryos have a reduced ability to regulate their internal pH (pH_i) and may be more susceptible to fluctuations in pH_e. With improper pH_e, the embryo could be stressed, in part, through impact on pH_i, which can impact metabolism and other intracellular processes.

Regarding aneuploidy, pH may impact stability and functioning of the oocyte meiotic spindle, which could possibly impact proper separation and segregation of chromosomes. While a prior preliminary study indicated no impact of a wide pH_e range covering 7.4–7.8 on presence/absence or size of the mouse meiotic spindle using polarized imaging (*Clark et al., 2011*), a more recent study indicated that altering pH_e of the medium impacted the retardance of the mouse oocyte meiotic spindle as assessed by polarized microscopy (*Swearman et al., 2018*). Thus, a similar mode of action of pH on mitotic spindles might be possible during embryo development; however, visually assessing this is difficult due to smaller size and timing. A prior study demonstrated that altered pH_i of hamster embryos can impact intracellular organization of organelles, such as mitochondria, but had no impact on microtubule structure (*Squirrell et al., 2001*). Function or resulting aneuploidy/mosaicism were not examined.

Preliminary data altering pH_e during different stages of human embryo culture has been shown to impact post-cleavage preimplantation embryo morphokinetic timings (*Adolfsson et al., 2016*). While mitotic cleavage timings themselves did not appear to be significantly impacted in the study, existing data indicate a possible correlation between embryo morphokinetics and aneuploidy (*Campbell et al., 2013; Huang et al., 2019; Kramer et al., 2014; Montag, 2013; Patel et al., 2016; Swain, 2013*).

As mentioned previously, when comparing two different media with pH of either 7.27 or 7.14, the medium with lower pH yielded embryos with higher rates of mitotic chromosome errors. Differences could not be attributed to pH alone (*Hickman et al., 2016*).

A preliminary study cultured frozen human two-pronuclear embryos (2PN) to the blastocyst stage and indicated that using an elevated pH and high oxygen (21%) during culture increased blastocyst mosaicism compared with control blastocysts that were cultured using a more appropriate pH and low oxygen (5%) (*Katz-Jaffe et al., 2018*). However, it is important to note that this study was retrospective in nature, and was performed over a long period of time with embryo culture performed in two different laboratories. Control blastocysts were cultured in an active clinical IVF laboratory and trophectoderm biopsies performed by taking 4–6 cells. Treatments using elevated pH and high oxygen were cultured in a research laboratory and genetic analysis performed by sampling the entire blastocyst cut into four equal portions. Sampling the entire blastocyst could be an explanation for why increased mosaicism was observed compared with blastocysts where only 4–6 cells were analysed.

Therefore, pH appears to be a logical variable within the culture system that could impact embryo mitotic spindle function and possibly chromosome dynamics. Preliminary data suggest a possible link but confounding variables preclude any conclusive evidence.

Oxygen

It has long been shown in a variety of animal models that use of reduced oxygen (around 5%) throughout the culture period is superior to atmospheric levels (around 21%) for embryo culture *in vitro*. Several prospective randomized trials demonstrate a similar benefit of reduced oxygen for human embryo culture when used during the entire period, including positive impact on live birth (*Kasterstein et al., 2013; Meintjes et al., 2009; Waldenstrom et al., 2009*). These data have been summarized in a systematic review and meta-analysis (*Bontekoe et al., 2012; Mantikou et al., 2013*), although others question those findings and the quality of the evidence (*Nastri et al., 2016*). Importantly, while some of the included studies indicate no beneficial impact for human embryo culture, often due to poor study design, limited endpoint assessment or when only culturing for 2–3 days, no studies have indicated a disadvantage to the approach.

Interestingly, a survey from 2014 indicates a significant number of IVF laboratories around the world appear to still utilize high oxygen for embryo culture (*Christianson et al., 2014*). This could possibly help explain the wide variations in aneuploidy reported between clinics.

In a mouse model, low oxygen (5%) used during embryo culture resulted in lower amounts of chromosome non-disjunction during early cleavage stage development, more similar to levels experienced *in vivo* (*Bean et al., 2002*). High oxygen (21%) culture yielded significantly higher rates of chromosomal errors.

A preliminary study retrospectively analysed the impact of high versus low oxygen when culturing human blastocysts. Culture was performed during different time periods in different incubators. Rates of mosaicism were high and differed, being around 4% lower in the low oxygen groups (37.3%) compared with the high oxygen (41.6%), but this was not statistically significant (*Black et al., 2018*). While unable to isolate the impact of oxygen levels alone, another preliminary study suggested that improper CO_2 and elevated O_2 (~21%) may increase blastocyst mosaicism, resulting in an ~17% higher rate of mosaicism (2.3% versus 20%) due to elevated pH and oxygen when compared with optimized culture conditions (*Katz-Jaffe et al., 2018*).

How elevated oxygen might impact embryo aneuploidy is not entirely clear.

TABLE 2 VARIABLES IMPACTING MEDIA EVAPORATION AND RESULTING MEDIA OSMOLALITY, WHICH MAY NEGATIVELY IMPACT SPINDLE FIDELITY AND INTERFERE WITH PROPER CHROMOSOME DYNAMICS

Laboratory procedure	Variables impacting media evaporation and resulting osmolality/pH increase	Reference
Dish preparation	Preparation time, temperature, humidity, air flow, drop size, microdrop preparation method	*Swain et al., 2012*
Culture period	Humidity of incubator, media drop size, amount of oil overlay, type of oil used, length of culture period, media exchange frequency	Olds, 2015; *Swain 2018; Swain et al., 2016, 2018*

Impact of reactive oxygen species, altered metabolism or other intracellular cell stress could impact on the function of key regulatory machinery involved with mitosis. Continued research into ultra-low oxygen or differential oxygen concentrations during embryo culture may yield further insight into the possible impact of oxygen levels on mitosis (*Kaser et al., 2018; Morin, 2017; Morin et al., 2017*).

Osmolality

Osmolality of culture medium impacts cell volume control and is a well-known cell stressor that can impact embryo development (*Baltz and Tartia, 2010; Swain et al., 2012*). Visually, the impact of osmolality is most apparent when transferring embryos through high osmolality vitrification solutions, where the cell shrinks, and back through warming solutions, where the cell re-expands. When performed with controlled exposure, gametes and embryos can handle certain osmolality shifts. However, in uncontrolled conditions or over extended periods, the negative impact becomes apparent.

Osmolality of culture medium can be inadvertently changed in the IVF laboratory during routine procedures where evaporation may occur (TABLE 2). Variables such as time, volume, temperature and air flow can impact evaporation during culture dish preparation and increase osmolality (*Swain et al., 2012*). The use of uninterrupted culture and non-humidified incubators, including time-lapse systems, can increase evaporation under certain conditions (*Fawzy et al., 2017; Swain et al., 2016*). In this instance dish type and volume of medium along with type of oil and amount of oil overlay are critical factors to avoid evaporation and detrimental osmolality increase over time (*Carpenter et al., 2018; Swain, 2018; Swain et al., 2016, 2018*). Importantly, evaporation also results in an additional stressor via an increase in medium pH (*Olds et al., 2015; Swain et al., 2016*).

Osmolality increases may be relevant to embryo aneuploidy. It has been shown that osmotic stress can impact fidelity of the meiotic spindle in human oocytes. For every 1 mOsm increase, the human oocyte meiotic spindle was disrupted by a factor of 1.001–1.004 (FIGURE 2) (*Mullen et al., 2004*). If a similar mechanism is present with the embryo mitotic spindle, this could impact chromosome dynamics and aneuploidy/mosaicism. However, such a mechanism has not yet been demonstrated during embryo development.

Temperature

Temperature is another important variable in the culture system that impacts embryo development and might impact embryo aneuploidy. The impact of cooling on the human oocyte and dispersion of the meiotic spindle is well documented (*Almeida and Bolton, 1995; Bernard et al., 1992; Pickering et al., 1990; Wang et al., 2001; Zenzes et al., 2001*). It is known that cooling the oocyte meiotic spindle can cause disassembly. However, the spindle is a dynamic structure. Using high-quality starting material and given adequate time, reassembly can successfully occur. Furthermore, outcome data from oocyte vitrification studies demonstrate that vitrification and warming oocytes does not increase aneuploidy compared with fresh oocytes (*Chamayou et al., 2017; Cobo et al., 2001; Forman et al., 2012; Goldman et al., 2015*). Importantly, most vitrification protocols entail holding the cells for several minutes at room temperature, both during the cryoprotectant exposure portion, and the final warming stages in the absence of cryoprotectants. Thus, in this context of a tested and controlled protocol, exposure to room temperature and to lower temperatures does not appear to induce severe levels of oocyte-derived meiotic aneuploidy. However, this does not necessarily mean that uncontrolled

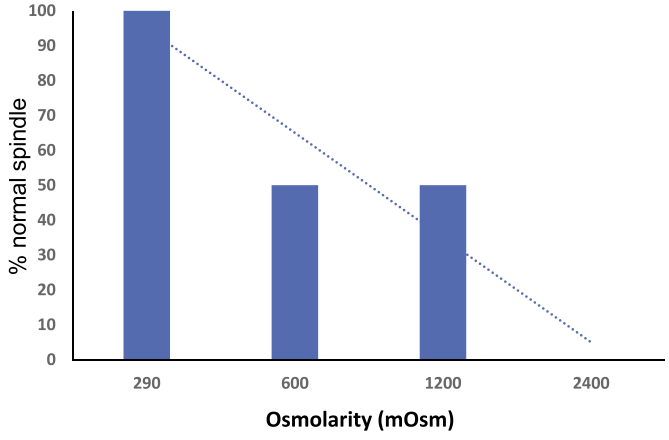

FIGURE 2 Increasing media osmolality disrupts the human oocyte meiotic spindle. For every 1 mOsm increase, the spindle is disrupted by an odds ratio of 1.001–1.004 (adapted from *Mullen et al., 2004*).

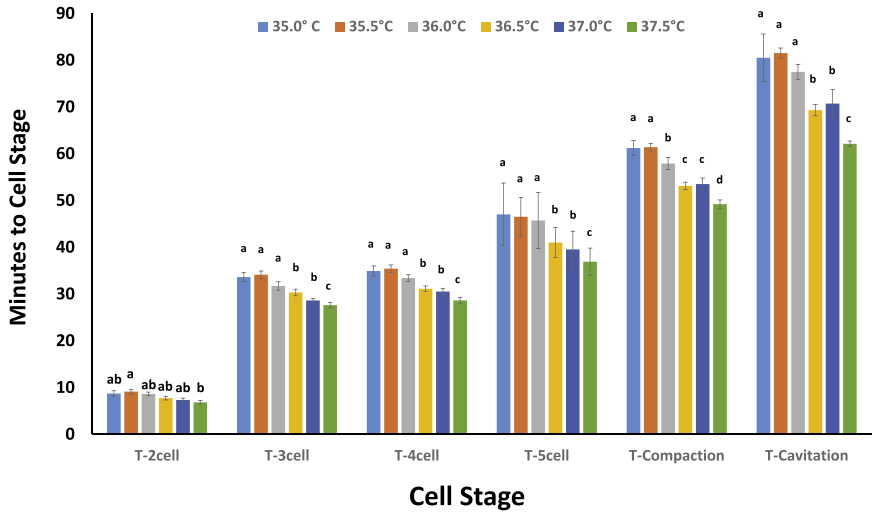

FIGURE 3 Impact of a temperature gradient on mouse embryo morphokinetic (mitotic division) timings and blastocyst development (*Walters et al., 2018*). Different superscripts within a stage represent statistically significant differences, $P < 0.05$.

or excessive, repeated decreases in temperature cannot have a more dramatic negative impact.

Whether temperature can influence mitotic errors in embryos has been less well studied. Although not isolated as the sole variable, temperature, as shown by lack of warming stages in one laboratory during one series, was postulated to be one of several possible laboratory factors responsible for varying rates of mosaicism between laboratories in cleavage-stage embryos analysed via FISH (*Munne et al., 1997*).

Varying temperature by even 0.5°C over an extended period of time certainly impacts mitosis and morphokinetic cell timings, as demonstrated in mouse embryos (*Walters et al., 2018*) (FIGURE 3). Morphokinetic timings have been correlated with embryo aneuploidy (*Campbell et al., 2013; Kramer et al., 2014; Montag, 2013; Patel et al., 2016; Swain, 2013*). This could imply that temperature could be impacting chromosome dynamics in the preimplantation embryo. Notably, in a well-designed prospective randomized controlled trial, significantly fewer cells were present by Day 3, indicating possibly fewer mitotic cell divisions at 36°C versus 37°C. However, no difference in blastocyst aneuploidy using was apparent (*Hong et al., 2014*) (TABLE 3). Rates of mosaicism were not reported.

No clear correlation between temperature and embryo mitotic errors is apparent. Importantly, maintenance of constant temperature within the IVF laboratory during embryo handling and culture is difficult (*Cooke et al., 2002*). Numerous factors can impact thermal stability and isolating temperature as a variable and exploring subtle impacts on chromosome dynamics becomes difficult.

LABORATORY CULTUREWARE/ PLASTICS

While toxicity testing of laboratory consumables is commonplace and a cornerstone of a good quality control programme, often overlooked, the plastics used in the IVF laboratory may have an impact on chromosome dynamics. Bisphenols are a common ingredient in plastics that can leach out and convey potentially harmful oestrogenic effects. Increases in meiotic aneuploidy have been reported from systemic bisphenol A (BPA) exposure in the mouse model (*Hunt et al., 2003*). Several reports exist on the negative impact of BPA on oocyte meiotic progression and disruption of the meiotic spindle (*Campen et al., 2018; Can et al., 2005; Machtinger et al., 2013*). However, it should be noted that at least one study showed that addition of BPA to mouse oocytes in cell culture had no impact on resulting aneuploidy (*Eichenlaub-Ritter et al., 2008*). Bisphenols have been shown to cause mitotic aneuploidy in Syrian hamster embryo cell culture (*Tsutsui et al., 1998, 2000*). Tubulin in embryonic and somatic cells appears to be a target of BPA (*George et al., 2008*). However, data are lacking on the impact of plastics/BPA in embryo mitotic errors/mosaicism. Fortunately, detectable levels of BPA have not been found in IVF culture media or protein supplements or leaching from common plastics used in the laboratory (*Gatimel et al., 2016; Mahalingaiah et al., 2012*), although levels are present in some items, like

TABLE 3 INFLUENCE OF A 1°C DIFFERENCE IN CULTURE TEMPERATURE ON HUMAN DAY 3 CELL NUMBER, USABLE BLASTOCYST AND ANEUPLOIDY RATES

Temperature	Day 3 cell #	Usable blastocyst rate (%)	% aneuploidy
36°C	7.0 ± 0.1[a]	41.2[a]	42.5
37°C	7.7 ± 0.1[b]	48.4[b]	46.1

Data from *Hong et al., 2014*.

Different superscripts between temperatures are statistically different, $P < 0.05$.

TABLE 4 RATES OF BLASTOCYST MOSAICISM BETWEEN SIX LABORATORIES USING THE SAME CULTURE SYSTEM/ CONDITIONS, STRICT QUALITY CONTROL PROCEDURES AND THE SAME GENETICS REFERENCE LABORATORY

Laboratory #	1	2	3	4	5	6
Mosaic rate (%)	2.6	2.4	2.4	2.5	2.1	2.8

Data from *Katz-Jaffe et al., 2017*.

polycarbonate stripper tips (*Gatimel et al., 2016*). Other contaminants or environmental stressors from plastics used in the laboratory could still possibly impact the spindle apparatus and chromosome dynamics. Thus, impact of common laboratory consumables should continue to be screened for toxicity and possible negative impact on aneuploidy/ mosaicism and in-vitro embryo developmental potential.

OTHER FACTORS

It must be noted that laboratory practices and techniques may also influence chromosomal testing results. Factors such as biopsy technique and cell handling may impact DNA integrity. Variances in method used to obtain trophectoderm cells, number of cells taken, location of cells taken, rigour of washing cells before loading, time or temperature of holding cells between biopsy and cell loading and shipping conditions of cells, could all potentially impact DNA quality and possibly fidelity of the preimplantation genetic testing for aneuploidies (PGT-A) results. Additionally, improved analytical platforms and increased sensitivity mean that carry-over and DNA contamination when processing cells must also be considered. Indeed, minute amounts of DNA extruded by embryos into culture media can be used to assess chromosomal complement (*Kuznyetsov et al., 2018; Liu et al., 2017; Vera-Rodriguez et al., 2018*). Thus, factors such as thoroughness of rinsing embryos prior to biopsy, or amount of medium carry-over when moving embryos, especially if cultured in groups, may impact genetic results. Factors such as medium carry-over if using the same biopsy pipette or loading pipette between embryos could be important considerations. Thus, the process of obtaining cells for analysis may be as likely a cause for observed differences between laboratories as the conditions used to grow the embryos. The influence of the genetics laboratory must also not be overlooked (*Laoannou et al., 2018*). Different genetics laboratories use different methods for cell loading and analysis, with some using more manual methods and others using more automation. Different platforms and thresholds are used for diagnoses of normal versus abnormal versus mosaic. Some result interpretations are performed manually, while others are automated. Emergence of artificial intelligence for interpreting genetic results is now used as well. Additionally, as some laboratories promote use of standard insemination, subtle contamination from insufficient removal of cumulus cells or excess bound spermatozoa interfere with PGT-A results enough to influence subtleties in mosaicism readings. A recent study indicates use of standard insemination may increase mosaic cells by 5% compared with use of ICSI (*Palmerola et al., 2019*), although these findings may be refuted due to unique sperm DNA packaging and if the laboratory ensures eggs/embryos are properly washed prior to biopsy.

It is clear that the number of variables involved with providing a high-quality sample for chromosomal analysis is immense. Interestingly, in a large network of IVF laboratories using the same culture system and protocols including the same medium, protein, oil, plasticware, pH, incubators, oxygen, biopsy training, quality management systems, and other relevant items, along with the same genetics laboratory, no differences in rates of mosaicism were seen between facilities (<3% total mosaicism per location with <1% variation between six locations) (*Katz-Jaffe et al., 2017*) (TABLE 4). Thus, while the patient population and stimulation protocols should not be overlooked, using stringent laboratory quality control, variability in aneuploidy/mosaicism between IVF facilities can be minimized.

CONCLUSION

It is important to note that while plausible cause-and-effect scenarios can be postulated for how variables within the embryo culture system may impact embryo mitosis and some preliminary data exist to suggest IVF laboratory-controlled culture variables may influence preimplantation embryo mitotic errors, concrete evidence is lacking. Cell stressors like pH, osmolality and temperature may all play a role with fidelity of mitotic divisions and, if not well controlled, subtle stress from each may act in a cumulative fashion to negatively impact fidelity of chromosome separation/segregation. Certainly, factors within the culture system controlling embryo development must be considered and continued research should focus on factors within the IVF laboratory that may lead to cellular stress and possible mitotic errors. However, the impact of laboratory technique (insemination approach, biopsy technique and other potential handling stressors), as well as the influence of the reference genetics laboratory, must not be overlooked.

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