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REVIEW

Controversies in ART: considerations and risks for uninterrupted embryo culture

BIOGRAPHY

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KEY MESSAGE

Optimized quality control and culture conditions are required to implement uninterrupted embryo culture. Media evaporation, with resulting osmolality and pH increases; media, protein and oil degradation, and volatile organic compound accumulation, could offset advantages of reduced dish and embryo handling. Incubator humidity, oil specifications, media, protein, and air and gas quality are important.

ABSTRACT

With new time-lapse incubators, IVF laboratories have increased use of single step media, often used in an uninterrupted approach. This simplifies the culture process for embryologists, may help reduce costs, offers the potential to reduce cell handling and associated harmful environmental stressors, and improves embryo quality and outcomes. One could argue, however, that optimized quality control and culture conditions are required to implement uninterrupted culture successfully. Without impeccable laboratory conditions and oversight, while trying to reduce harmful environmental stress, the laboratory could be imparting stress. Factors such as medium evaporation and associated osmolality and pH increases, as well as volatile organic compound accumulation, could offset any advantage of reduced dish or embryo handling. When implementing uninterrupted embryo culture, attention must be paid to incubator humidity, amount, quality and type of oil used, medium formulation and protein quality, as well as laboratory air and gas supply quality, and volatile organic compound content.

INTRODUCTION

Immense progress has been made in assisted reproductive technology (ART) over the past 40 years, resulting in increased success rates. Many of these advancements have occurred as a result of improvements within the IVF laboratory, where a continuous push has been made to further improve the culture system (*Niederberger et al., 2018*). This has been accomplished through research aimed at gaining a better understanding of the physiology of gametes and preimplantation embryos, and has manifested as improved media, customized equipment and novel procedures for handling and assessing quality and potential of the reproductive cells. Recently, the IVF laboratory has seen an increase in the use of single-step media made to grow embryos to the blastocyst stage and implementation of uninterrupted culture, with most media companies now offering a single-step product in their portfolio.

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KEYWORDS

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BENEFITS OF UNINTERRUPTED EMBRYO CULTURE

No clear consensus has been reached on the benefits of uninterrupted embryo culture (*Reed et al., 2009; Costa-Borges et al., 2016; Sfontouris et al., 2016; Alhelou et al., 2018*), often because of confounding variables; however, the potential benefits are readily apparent (TABLE 1). This approach reduces handling of the embryos. Reduced handling and removal of dishes from the confines of the incubator presumably results in more stable gas levels (pH) and temperature. Furthermore, reduced handling helps avoid potential sheer stress from pipetting and other associated risks with transporting embryos across the laboratory or between dishes. Additionally, continuous culture may result in accumulation of beneficial autocrine and paracrine factors from embryos that can improve development (*Reed et al., 2011; Swain and Smith, 2011; Smith et al., 2012*). Importantly, uninterrupted culture is compatible with new time-lapse imaging incubators to permit assessment of morphokinetic markers and other attributes of embryo development not previously feasible with manual observations, which may improve embryo selection. Furthermore, reduced handling of cells, media and dish exchanges, in conjunction with the ability to monitor cell development via a recorded video or series of images, may help with laboratory workflow and reduce staff workloads.

TABLE 1 POTENTIAL BENEFITS AND RISKS OF AN UNINTERRUPTED EMBRYO CULTURE PARADIGM

Benefits	*Risks*
Reduced dish removal from incubator: more stable culture conditions (gas, temperature)	Media degradation (ammonia production, substrate depletion and other component degradation)
Reduced cell handling: reduced risk for cell damage or loss	Missing possible important embryo morphological indicators or signs of potential culture issues (only in non-time lapse incubators)
Accumulation of beneficial autocrine and paracrine factors	Volatile organic compound accumulation (in oil and or media)
Compatible with new time-lapse incubators: new technology and additional selection end-points	Media evaporation: osmolality increase, pH increase and increase in other solute concentrations (in dry incubators)
Improved workflow: less staff time and possible cost savings	Mineral oil degradation (peroxidation)

SINGLE-STEP MEDIA

Without the existence of a clinically reliably microfluidic-type culture system to avoid manual handling of dishes for media exchange (*Smith et al., 2011; 2012*), implementation of uninterrupted culture also likely means use of a single-step medium. Comparisons of efficacy of single-step media with sequential media for culture of human embryos are fairly abundant and have been extensively reviewed (*Mantikou et al., 2013; Swain 2015; Youssef et al., 2015; Sfontouris et al., 2016; Dieamant et al., 2017*). Both sequential and single-step media can yield similar blastocyst, clinical pregnancy and live birth rates. No well-designed, prospective randomized controlled trials have compared outcomes between modern single-step medium, designed to culture embryos to the blastocyst stage, with sequential culture media. Evidence on whether single-step media may affect fidelity of mitotic chromosome dynamics, compared with sequential media in preimplantation embryos, is conflicting (*Werner et al., 2016; Fragouli et al., 2017; Vermilya et al., 2018*). On the basis of emerging data about the possible effect of culture media on offspring weight and cardiovascular health (*Kleijkers et al., 2014; Zandstra et al., 2015; 2018*), it is prudent that this remains an active area of research. Interestingly, single-step media did not seem to differ in the maintenance of genomic imprinting in the mouse embryo compared with their sequential media counterparts (*Market-Velker et al., 2010*). Several existing comparative studies, however, make it difficult to determine the effect and efficacy of the single-step medium or the uninterrupted approach compared with the effect of other culture system variables, e.g. patient, reduced handling of cells, different dishes, different incubator, different embryo selection and different gas concentrations. Nevertheless, high success rates can be achieved with various single-step culture media.

KEY CULTURE SYSTEM CONSIDERATIONS FOR UNINTERRUPTED CULTURE

Key variables must be considered with using a single-step medium in an uninterrupted fashion to avoid potential detrimental effects on developing preimplantation embryos (TABLE 1). As mentioned, failure to control these variables may explain some of the contradicting data on the efficacy of the uninterrupted culture paradigm.

Evaporation

Many modern embryo culture incubators now include smaller, individualized chambers to expedite recovery of temperature and gas to help maintain stability of the culture environment (*Swain, 2014*). Many of these new incubators lack humidity. The aim of removing humidity in modern embryo culture incubators, including most time-lapse systems, is to reduce contamination associated with moisture in the incubator. The improved design, function and maintenance of modern embryo culture incubators are also logistically beneficial. As a result, with no humidity, evaporation of culture media may

TABLE 2 LABORATORY CONSIDERATIONS FOR IMPLEMENTING UNINTERRUPTED EMBRYO CULTURE TO AVOID POSSIBLE RISKS

Oil volume (dish shape and size)
Oil density
Oil quality
Media volume
Gas supply and air quality (volatile organic compounds content)
Incubator and room humidity
Media type (substrates levels, amino acid content and formulation, osmolality)
Protein type (ammonia content, production and accumulation)

occur if conditions are not optimized to avoid this (*Mori et al., 2010*).

Recent studies indicate that human embryo development may be compromised in a dry incubator compared with a humidified incubator. Using patient splits, culture for 5 to 6 days in a single step-medium with no replenishment demonstrated that use of dry chambers yielded lower day-3 embryo development, lower blastocyst formation and reduced clinical pregnancy and implantation rates compared with humidified chambers (*Fawzy et al., 2017*). Studies were conducted in a dry climate and ambient humidity was not reported, nor was humidity achieved in either dry or humidified chambers. Additionally, clinical outcome rates were low in both groups, and the incubator used was made for dry incubation.

In a separate preliminary study, a novel time lapse incubator with six chambers, permitting either humidified or dry culture, offered a unique research tool for examining the potential effect of humidification under specific culture conditions more closely. Three chambers in the same incubator were prospectively randomized to a humidified group and three to a dry group. Importantly, humidity levels were not reported for any of the chambers. Undisturbed culture of human embryos from 83 patients in the dry chambers had lower blastocyst development and slower development to the five-cell stage and indicators of higher oxidative stress than culture of 93 other patients in an identical fashion (same medium, dish, oil) in humidified chambers (*Del Gallego et al., 2018*). Patient demographics were not reported to assess whether the populations studied may explain observed differences. Although not measured in these two studies, the observed results may be due, in part, to excessive evaporation in the dry chambers. Subsequent measurements in another laboratory indicate that humidity levels achieved while attempting to humidify a normally dry incubator may approach around 40–45% (*Holmes and Swain, 2018*), and evaporation will still occur, but at a slower rate than in a completely 'dry' chamber. This is similar to that observed in a humidified isolette with around 40–45% humidity (FIGURE 1).

Unsurprisingly, the longer the medium remains in the incubator, the more evaporation occurs. Therefore, when equilibrating media and culturing embryos for 5–7 days in a single-step medium in an uninterrupted fashion, culture dishes are exposed to evaporation conditions for upwards of 180 h. Factors such as volume and drop shape and size can affect rate of evaporation (*Iwata et al., 2016; Swain et al., 2018*). This is likely due to the amount of oil that sits above the medium, as it has been shown that 3 ml of oil results in more evaporation compared with 5 or 7 ml in the same sized dish (*Carpenter et al., 2018*). Importantly, media can evaporate under mineral oil (*Swain et al., 2016; 2018*) (FIGURE 2). Therefore, the amount of oil is critical to avoid these issues (*Olds et al., 2015; Carpenter et al., 2018, Swain et al., 2018*). The type of oil also affects the evaporation rate, with heavier and denser oil reducing evaporation compared with a lighter oil. Indeed, a difference in density of 0.04 g/ml can have a significant affect on evaporation (*Swain, 2018*) (FIGURE 3). Therefore, use of a heavier, denser oil may benefit evaporation for uninterrupted culture in a dry environment. Nomenclature of many commercial IVF oils is unclear, and it is difficult to determine the densities of commercial embryo culture oils for comparison. Presence of lids on dishes (FIGURE 3) or washing of oil (FIGURE 4) in dry incubators did not affect rate of media evaporation and resulting osmolality increase (*Swain et al., 2016*). Coupled with the prior data indicating that adding more oil overlay reduces osmolality increase demonstrates that the water portion of culture media is likely not being absorbed into the oil and that evaporation through the oil is actually occurring.

Evaporation results in an increase is medium osmolality, which affects cell volume control and can be a cell stressor and impair embryo development (*Baltz and Tartia, 2010; Baltz and Zhou 2012; Moravek et al., 2012; Swain et al., 2012*). Osmolality measuring over 300 mOsm/kg can inhibit embryo development, although the presence of amino acids in media act as osmolytes

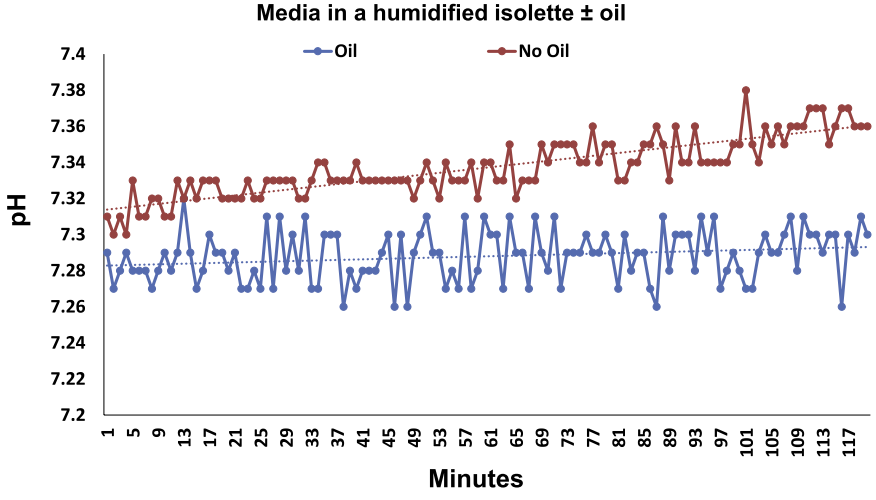

FIGURE 1 Media evaporation during embryo culture results in an increase in media pH caused by increased sodium bicarbonate concentration (*Swain et al., 2015; Holmes and Swain 2018*). Even in a semi-humidified environment, such as in a modified isolette or a culture incubator (~40–45% humidity), media evaporation occurs (data from *Swain et al., 2015*). This can be lessened, but not alleviated, via oil overlay. Evaporation increases over time (graph represents data collected from measurements within in a modified isolette chamber).

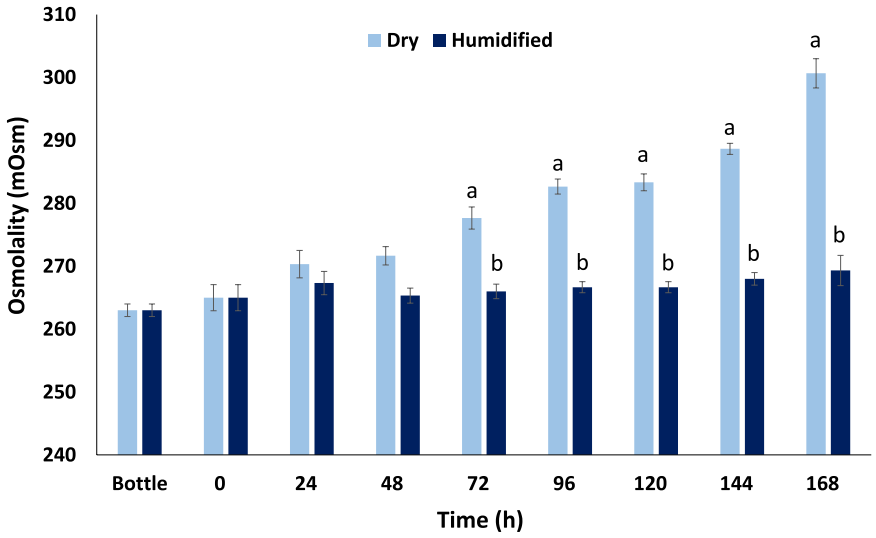

FIGURE 2 Culture media can evaporate while under mineral oil, as indicated by rising media osmolality. This is exacerbated by use of dry incubators and increases over time (data from *Swain et al., 2016*). Data show media osmolality over time after incubation, presented as the mean ± SEM. Significant differences occur between dry and humidified (>90% humidity) incubators by around 72 h (different superscripts between treatments within a time point indicate significant differences; $P < 0.05$). Conditions used were a 35-mm dish with 25-µl media drops covered in 3 ml of paraffin oil.

and can influence this. Therefore, starting osmolality and amino acid content is an important consideration with single-step media used for uninterrupted culture in a non-humidified environment, with starting osmolality recommended in the 255–265 mOsm/kg range, rather than media with osmolality in the range of 275–290 mOsm/kg.

Importantly, with evaporation, pH of culture media also increases. In a humidified isolette, with about 40% humidity and no oil overlay, evaporation occurs over time (*Swain et al., 2015; 2016*). This resulted in increased osmolality and also pH (*Swain et al., 2015; 2016*) (FIGURE 1). A similar trend was observed in a normally non-humidified incubator that was humidified to 40–50% (*Holmes and Swain, 2018*). This increase in pH with evaporation was caused by the resulting increase in concentration of sodium bicarbonate. Additionally, all other solute concentrations also increase when evaporation occurs. Therefore, when media evaporation occurs, the embryos are not exposed to the same components or concentrations as originally designed.

This evaporation of media during culture in dry incubators varies on the basis of size of media drop (*Iwata et al., 2016; Swain et al., 2018*). This is most likely a result of how flat the drop becomes, with larger drops layer flatter. Also important is the amount of oil used (*Olds et al., 2015; Swain et al., 2018*), specifically the amount of oil over the top of the media drop. More oil reduced media evaporation. Media evaporation can also occur in modern embryo culture dishes made for time-lapse culture incubators, with different dishes yielding differing amounts of evaporation based on the

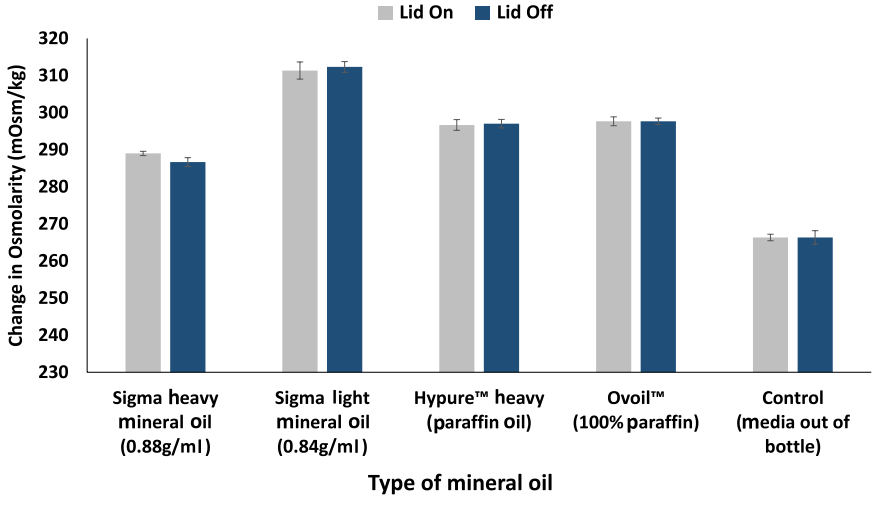

FIGURE 3 Type of mineral oil can affect rate of media evaporation. Data show media osmolality after incubation over time, presented as the mean ± SEM. Oils with higher density result in lower rates of media evaporation compared with lighter oils (data from *Swain, 2018*) (conditions presented were 25 µl drops under 3.5 ml of paraffin mineral oil in a 35 mm culture dish in a dry incubator for 144 h). Presence or absence of lid had no effect on rate of evaporation under these conditions.

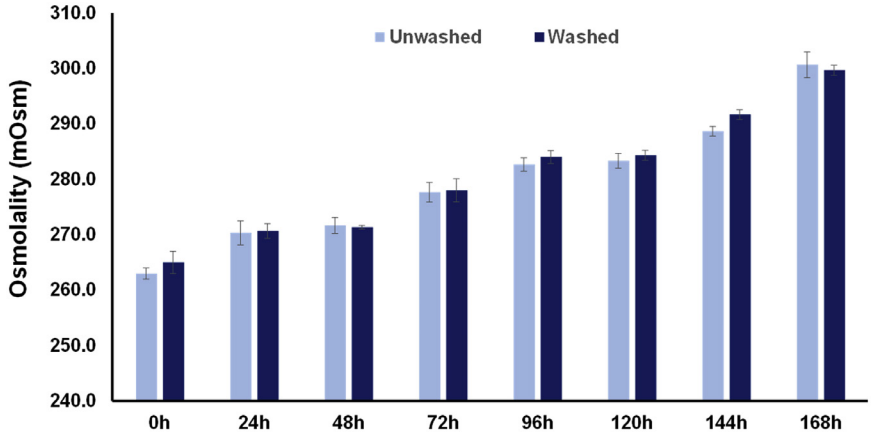

FIGURE 4 Washing of 100% paraffin oil has no effect on resulting media evaporation during extended culture in a dry incubator (data from *Swain et al., 2016*). Data show media osmolality after incubation over time, presented as the mean ± SEM. Conditions included a 35-mm dish with 25-µl media drops covered in 3 ml of paraffin oil.

variables mentioned above (*Carpenter et al., 2018*)

Under appropriate conditions, use of dry incubators can yield high embryo development and IVF outcomes. Use of single step media and uninterrupted culture can also be used successfully. Simply applying a prior culture system (dish type, media type, drop size, oil type, oil amount) that was successful in a humidified incubator environment or with media replenishment, however, may not yield the same results in a dry incubator if certain criteria are not first validated or verified. Simple precautions, such as measuring pH or osmolality or assessing changes in solute concentrations (which can be done using a blood gas analyzer) before clinical implementation of the approach, can yield insight into possible evaporation issues and permit the laboratory to optimize their culture system variables to avoid evaporation issues.

Volatile organic compounds

With prolonged exposure of a culture dish with oil overlay to the confines of the incubator during uninterrupted culture comes the potential for accumulation of harmful volatile organic compounds (VOC). It is known that VOC can be present within tanks of medical gases used for embryo culture (*Hall et al., 1998*). Therefore, some VOC may pass into, and accumulate within, the incubator where they tend to accumulate in mineral oil overlays and affect culture media and embryo development (*Martinez et al., 2017*). Volatile organic compounds that present in room air may also accumulate within the culture system, especially if using high oxygen culture, where room air fills the bulk of the incubator chamber. Therefore, particularly with extended, uninterrupted culture of embryos, proper precautions should be taken to ensure appropriately low VOC levels with the laboratory and inside the incubator specifically (*Mortimer et al., 2018*). Fortunately, most modern IVF laboratories use some sort of air filtration to reduce VOC in room air. Modern embryo culture incubators are often built to use low oxygen culture, and VOC filters can be applied to gas lines or come as a component of the incubator itself. Despite these measures, however, many laboratory filtration systems, carbon filters used in gas lines or in incubators are incapable of capturing all VOC. Future improvements in incubator technologies, including use of additional type of chemical filtration, such as potassium permanganate or other methods of VOC reduction, such as photocatalytic oxidation, may help alleviate potential VOC concerns within the confines of the embryo culture incubator.

Media degradation

Medium components can degrade over time during culture at elevated temperatures. With improper culture media, an additional 18 h of culture at 37°C during media equilibration before the presence of embryos can negatively affect embryo development (*Bavister and Poole, 2005*). Therefore, degradation needs to be a consideration when using media in an uninterrupted fashion.

A potential advantage of sequential culture media approach is the change of the medium to meet the metabolic needs of the developing embryos. This results in replenishment of nutrients to avoid possible depletion, provides specific substrates at specific time points and aids in and of byproducts, such as ammonia. Generation of ammonia, primarily from the amino acid glutamine, can inhibit embryo development and cause fetal defects (*Gardner and Lane, 1993; Lane and Gardner, 1994; Hammon et al., 2000; Lane and Gardner, 2003; Virant-Klun et al., 2006*). Modern embryo culture media, however, including single-step systems, have reduced the amino acid content and also use the more stable dipeptide forms of glutamine (*Biggers et al., 2004*). These adjustments result in only low levels of ammonia being produced, even during uninterrupted culture with up to five embryos present from 5–7 days (43–84 µM) (*Gilbert et al., 2012*), less than the around 120µM shown to inhibit human blastocyst formation (*Virant-Klun et al., 2006*), and can even yield lower levels of ammonia (<25 µM without embryos) than some sequential media systems used at around 72 intervals (*Hardarson et al., 2015*). Different protein sources, however, can also generate differing amounts of ammonia (*Kleijkers et al., 2016*). In pre-supplemented media studied, most ammonia produced over 4 days of incubation at 37°C seemed to be generated from the protein supplement, rather than the culture medium. The same held true for storage for 6 weeks at 2–8°C. The amount of ammonia generated by the protein supplements studied varied by as much as 125 µM after 4 days of culture at 37°C, although

concentration of the proteins studied needs to be considered. Interestingly, the lowest ammonia production came from a recombinant albumin protein product, whereas the highest was generated by a complex protein supplement with albumin plus globulins. Another complex protein supplement, however, yielded lower levels of ammonia, similar to other albumin products. Therefore, protein source and type should be considered when implementing an uninterrupted system where accumulation of ammonia over time may be a concern. It may be advantageous for manufacturers of single-step media who recommended uninterrupted use of their product to perform and report degradation studies, including reporting ammonia levels, especially if media are pre-supplemented, making sure to also measure and report on the effect of recommended and compatible protein supplements in their quality control literature.

Mineral oil degradation

In addition to possible degradation of media or protein caused by extended culture at 37°C, culture of oil at elevated temperature over time may also present issues. Mineral oil peroxidation negatively affects media and embryo development (*Otsuki et al., 2007; 2009; Martinez et al., 2017*). Both time and elevated temperature can increase peroxidation of mineral oil (*Otsuki et al., 2007; 2009*). Mineral oil with an undetectable peroxide level showed increased peroxide levels during culture at 50°C over 20 days (0.04 mEq/kg) and 50 days (0.1 mEg/kg) (*Otsuki et al., 2007*). Simply opening a bottle of oil resulted in increased peroxidation after several weeks compared with unopened oil (*Otsuki et al., 2007*). Initial peroxide content of oils vary, and storage of oil at room temperature for 4 or 12 months tended to increase peroxidation levels compared with storage at 2–8°C. (*Otsuki et al., 2009*). Although this temperature and extended timelines may not be of concern to most laboratories practising IVF, preliminary data suggest that increase peroxide levels, sufficient enough to impair embryo development, may be achieved under laboratory conditions (0.3 mEq/kg after 96 h of culture at 37°C) (*Inoue, 2017*). Therefore, the initial quality of the oil is likely to be important to ensure damaging peroxides are not present before culture. This starting oil quality, including VOC content, peroxide levels and ionic composition, all which may differ between oils (*Martinez et al., 2017*), may be more important if using an uninterrupted approach and thorough oil quality testing and improved reporting by manufacturers and distributors is prudent (*Hughes et al., 2010; Morbeck et al., 2010; Khan et al., 2012; Wolff et al., 2013; Ainsworth et al., 2017*).

In conclusion, the field of IVF has undergone a shift to more laboratories using modern embryo culture incubators, many of which are non-humidified. A shift in use of single step media and an uninterrupted culture approach has also taken place. This simplifies the process for embryologists, may help reduces costs and is compatible with emerging time-lapse technologies. This approach of uninterrupted embryo culture offers the potential to reduced harmful environmental stressors and improve embryo quality and outcomes. One could argue, however, that optimized quality control and culture conditions are required to successfully implement uninterrupted culture. Without impeccable laboratory conditions and oversight, while implementing uninterrupted culture to try to reduce harmful environmental stress, the laboratory could be imparting stress. Factors such as evaporation and associated osmolality and pH increases, as well oil peroxidation or VOC accumulation, could offset any advantage of reduced dish and embryo handling. If implementing single-step media with uninterrupted culture, even within new time lapse incubators, attention must be paid to other culture system variables to avoid unwanted negative outcomes. Proposed best practice approaches to avoid unwanted issues when using an uninterrupted culture paradigm are presented in **TABLE 3**.

TABLE 3 PROPOSED BEST PRACTICES OR CONSIDERATIONS IF IMPLEMENTING OR USING UNINTERRUPTED EMBRYO CULTURE

Use of humidified incubation if possible, especially if culturing to the blastocyst stage, but not required if other variables are optimized.
Monitoring of consistent ambient room humidity (~30–50% recommended)
Use of appropriate medium with a starting osmolality ~255–270 containing the dipeptide form of glutamine (alanyl- or glycyl-)
Use of appropriate medium volume and sufficient oil overlay
Use of appropriate oil type (paraffin or heavy oil)
Use of high-quality oil (low peroxide and volatile organic compounds levels)
Use of volatile organic compounds filtration for laboratory air, medical gas supply and incubator recirculation
Use of high-quality protein supplements with low or no ammonia, and low accumulation
Measurement of medium characteristics (pH, osmolality and electrolytes) before and after uninterrupted culture up to 7 days under the laboratory conditions used to confirm adequacy of the culture system before culturing human embryos. Re-measurement after any changes to the culture system. Adjustments to the culture system can be made if differences in end-point assessments are noted.

REFERENCES

Ainsworth, A. J., Fredrickson, J. R., Morbeck, D. E. **Improved detection of mineral oil toxicity using an extended mouse embryo assay.** J. Assist. Reprod. Genet. 2017; 34: 391–397

Alhelou, Y., Mat Adenan, N. A., Ali, J. **Embryo culture conditions are significantly improved during uninterrupted incubation: A randomized controlled trial.** Reprod. Biol. 2018; 18: 40–45

Baltz, J. M., Tartia, A. P. **Cell volume regulation in oocytes and early embryos: connecting physiology to successful culture media.** Hum. Reprod. Update 2010; 16: 166–176

Baltz, J. M., Zhou, C. **Cell volume regulation in mammalian oocytes and preimplantation embryos.** Mol. Reprod. Dev. 2012; 79: 821–831

Bavister, B. D., Poole, K. A. **Duration and temperature of culture medium equilibration affect frequency of blastocyst development.** Reprod. Biomed. Online 2005; 10: 124–129

Biggers, J. D., McGinnis, L. K., Summers, M. C. **Discrepancies between the effects of glutamine in cultures of preimplantation mouse embryos.** Reprod. Biomed. Online 2004; 9: 70–73

Carpenter, G., Hammond, E., Peek, J., Morbeck, D. **The impact of dry incubation on osmolality of media in time lapse culture dishes.** Hum. Reprod. 2018; 33: i61

Costa-Borges, N., Belles, M., Meseguer, M., Galliano, D., Ballesteros, A., Calderon, G. **Blastocyst development in single medium with or without renewal on day 3: a prospective cohort study on sibling donor oocytes in a time-lapse incubator.** Fertil. Steril. 2016; 105: 707–713

Del Gallego, R., Albert, C., Marcos, J., Larreategui, Z., Alegre, L., Meseguer, M. **Humid vs. dry embryo culture conditions on embryo development: a continous embryo monitoring assessment.** Fertil. Steril. 2018; 110: e362

Dieamant, F., Petersen, C. G., Mauri, A. L., Comar, V., Mattila, M., Vagnini, L. D., Renzi, A., Petersen, B., Ricci, J., Oliveira, J. B. A., Baruffi, R. L. R., Franco, J. G. Jr. **Single versus sequential culture medium: which is better at improving ongoing pregnancy rates? A systematic review and meta-analysis.** JBRA Assist. Reprod. 2017; 21: 240–246

Fawzy, M., AbdelRahman, M. Y., Zidan, M. H., Abdel Hafez, F. F., Abdelghafar, H., Al-Inany, H., Bedaiwy, M. A. **Humid versus dry incubator: a prospective, randomized, controlled trial.** Fertil. Steril. 2017; 108: 277–283

Fragouli, E., Alfarawati, S., Simkins, M., Cutts, G., Spath, K., Babariya, D., Kubikova, N., Rubistello, L., Munne, S., Wells, D. **Factors affecting embryonic mosaicism.** Human Reproduction 2017; 32: i49

Gardner, D. K., Lane, M. **Amino acids and ammonium regulate mouse embryo development in culture.** Biol. Reprod. 1993; 48: 377–385

Gilbert, R., Hwan, S., Vermilyea, M., Graham, J., Tucker, M. **Low levels of ammonium detected in spent media from mouse or human embryos cultured in continuous culture (CSC) medium are not detrimental to development.** Fertil. Steril. 2012; 98: s169

Hall, J., Gilligan, A., Schimmel, T., Cecchi, M., Cohen, J. **The origin, effects and control of air pollution in laboratories used for human embryo culture.** Hum. Reprod. 1998; 13: 146–155

Hammon, D. S., Wang, S., Holyoak, G. R. **Effects of ammonia during different stages of culture on development of in vitro produced bovine embryos.** Anim. Reprod. Sci. 2000; 59: 23–30

Hardarson, T., Bungum, M., Conaghan, J., Meintjes, M., Chantilis, S. J., Molnar, L., Gunnarsson, K., Wikland, M. **Noninferiority, randomized, controlled trial comparing embryo development using media developed for sequential or undisturbed culture in a time-lapse setup.** Fertil. Steril. 2015; 104

Holmes, R., Swain, J. **Humidification of a dry benchtop IVF incubator: impact on culture media parameters.** Fertil. Steril. 2018; 110: e52

Hughes, P. M., Morbeck, D. E., Hudson, S. B., Fredrickson, J. R., Walker, D. L., Coddington, C. C. **Peroxides in mineral oil used for in vitro fertilization: defining limits of standard quality control assays.** J. Assist. Reprod. Genet. 2010; 27: 87–92

Inoue, F. **Effect of antioxidant addition to oil for embryo culture.** Fertil. Steril. 2017; 108: e60

Iwata, K., Yumoto, K., Mio, Y. **Unstable osmotic pressure in microdrops cultured under mineral oil in non-humidifed incubators.** Fertil. Steril. 2016; 106: e355

Khan, Z., Wolff, H. S., Fredrickson, J. R., Walker, D. L., Daftary, G. S., Morbeck, D. E. **Mouse strain and quality control testing: improved sensitivity of the mouse embryo assay with embryos from outbred mice.** Fertil. Steril. 2012

Kleijkers, S. H., van Montfoort, A. P., Bekers, O., Coonen, E., Derhaag, J. G., Evers, J. L., Dumoulin, J. C. **Ammonium accumulation in commercially available embryo culture media and protein supplements during storage at 2-8 degrees C and during incubation at 37 degrees C.** Hum. Reprod. 2016; 31: 1192–1199

Kleijkers, S. H., van Montfoort, A. P., Smits, L. J., Viechtbauer, W., Roseboom, T. J., Nelissen, E. C., Coonen, E., Derhaag, J. G., Bastings, L., Schreurs, I. E., Evers, J. L., Dumoulin, J. C. **IVF culture medium affects post-natal weight in humans during the first 2 years of life.** Hum. Reprod. 2014; 29: 661–669

Lane, M., Gardner, D. K. **Increase in postimplantation development of cultured mouse embryos by amino acids and induction of fetal retardation and exencephaly by ammonium ions.** J. Reprod. Fertil. 1994; 102: 305–312

Lane, M., Gardner, D. K. **Ammonium induces aberrant blastocyst differentiation, metabolism, pH regulation, gene expression and subsequently alters fetal development in the mouse.** Biol. Reprod. 2003; 69: 1109–1117

Mantikou, E., Youssef, M. A., van Wely, M., van der Veen, F., Al-Inany, H. G., Repping, S., Mastenbroek, S. **Embryo culture media and IVF/ICSI success rates: a systematic review.** Hum. Reprod. Update 2013; 19: 210–220

Market-Velker, B. A., Fernandes, A. D., Mann, M. R. **Side-by-side comparison of five commercial media systems in a mouse model: suboptimal in vitro culture interferes with imprint maintenance.** Biol. Reprod. 2010; 83: 938–950

Martinez, C. A., Nohalez, A., Ceron, J. J., Rubio, C. P., Roca, J., Cuello, C., Rodriguez-Martinez, H., Martinez, E. A., Gil, M. A. **Peroxidized mineral oil increases the oxidant status of culture media and inhibits in vitro porcine embryo development.** Theriogenology 2017; 103: 17–23

Martinez, C. A., Nohalez, A., Parrilla, I., Motas, M., Roca, J., Romero, I., Garcia-Gonzalez, D. L., Cuello, C., Rodriguez-Martinez, H., Martinez, E. A., Gil, M. A. **The overlaying oil type influences in vitro embryo production: differences in composition and compound transfer into incubation medium between oils.** Sci. Rep. 2017; 7: 10505

Moravek, M., Fisseha, S., Swain, J. E. **Dipeptide forms of glycine support mouse preimplantation embryo development in vitro and provide protection against high media osmolality.** J. Assist. Reprod. Genet. 2012; 29: 283–290

Morbeck, D. E., Khan, Z., Barnidge, D. R., Walker, D. L. **Washing mineral oil reduces contaminants and embryotoxicity.** Fertil. Steril. 2010; 94: 2747–2752

Mori, C., Kuwayama, M., Silber, S., Kagawa, N., Kato, H. **Water evaporation and osmolality change of human embryo culture media in humid or in dry culture systems.** Fertil. Steril. 2010; 94: s151

Mortimer, D., Cohen, J., Mortimer, S. T., Fawzy, M., McCulloh, D. H., Morbeck, D. E., Pollet-Villard, X., Mansour, R. T., Brison, D. R., Doshi, A., Harper, J. C., Swain, J. E., Gilligan, A. V. **Cairo consensus on the IVF laboratory environment and air quality: report of an expert meeting.** Reprod. Biomed. Online 2018; 36: 658–674

Niederberger, C., Pellicer, A., Cohen, J., Gardner, D. K., Palermo, G. D., O'Neill, C. L., Chow, S., Rosenwaks, Z., Cobo, A., Swain, J. E., Schoolcraft, W. B., Frydman, R., Bishop, L. A., Aharon, D., Gordon, C., New, E., Decherney, A., Tan, S. L., Paulson, R. J., Goldfarb, J. M., Brannstrom, M., Donnez, J., Silber, S., Dolmans, M. M., Simpson, J. L., Handyside, A. H., Munne, S., Eguizabal, C., Montserrat, N., Izpisua Belmonte, J. C., Trounson, A., Simon, C., Tulandi, T., Giudice, L. C., Norman, R. J., Hsueh, A. J., Sun, Y., Laufer, N., Kochman, R., Eldar-Geva, T., Lunenfeld, B., Ezcurra, D., D'Hooghe, T., Fauser, B., Tarlatzis, B. C., Meldrum, D. R., Casper, R. F., Fatemi, H. M., Devroey, P., Galliano, D., Wikland, M., Sigman, M., Schoor, R. A., Goldstein, M., Lipshultz, L. I., Schlegel, P. N., Hussein, A., Oates, R. D., Brannigan, R. E., Ross, H. E., Pennings, G., Klock, S. C., Brown, S., Van Steirteghem, A., Rebar, R. W., LaBarbera, A. R. **Forty years of IVF.** Fertil. Steril. 2018; 110

Olds, S., Stemm, K., Wachter, K., Wiemer, K. **Analysis of embryo culture media pH changes during incubator use and media evaporation under oil using a continous pH monitoring system.** Fertil. Steril. 2015; 104: e318–e319

Otsuki, J., Nagai, Y., Chiba, K. **Peroxidation of mineral oil used in droplet culture is detrimental to fertilization and embryo development.** Fertil. Steril. 2007; 88: 741–743

Otsuki, J., Nagai, Y., Chiba, K. **Damage of embryo development caused by peroxidized mineral oil and its association with albumin in culture.** Fertil. Steril. 2009; 91: 1745–1749

Reed, M., Woodward, B., Swain, J. **Single or group culture of mammalian embryos: the verdict of the literature.** J. Reprod. Biotech. Fertil. 2011; 2: 77–87

Reed, M. L., Hamic, A., Thompson, D. J., Caperton, C. L. **Continuous uninterrupted single medium culture without medium renewal versus sequential media culture: a sibling embryo study.** Fertil. Steril. 2009; 92: 1783–1786

Sfontouris, I. A., Martins, W. P., Nastri, C. O., Viana, I. G., Navarro, P. A., Raine-Fenning, N., van der Poel, S., Rienzi, L., Racowsky, C. **Blastocyst culture using single versus sequential media in clinical IVF: a systematic review and meta-analysis of randomized controlled trials.** J. Assist. Reprod. Genet. 2016; 33: 1261–1272

Smith, G. D., Swain, J. E., Bormann, C. L. **Microfluidics for gametes, embryos, and embryonic stem cells.** Semin. Reprod. Med. 2011; 29: 5–14

Smith, G. D., Takayama, S., Swain, J. E. **Rethinking in vitro embryo culture: new developments in culture platforms and potential to improve assisted reproductive technologies.** Biol. Reprod. 2012; 86: 62

Swain, J. **Different mineral oils used for embryo culture microdrop overlay differentially impact media evaporation.** Fertil. Steril. 2018; 109: e53

Swain, J., Batcheller, A., Schoolcraft, W., Bossert, N. **Washing mineral oil used for microdrop overlay does not improve stability of media osmolality.** Fertil. Steril. 2016; 106: e355

Swain, J., Graham, C., Kile, R., Schoolcraft, W. B., Krisher, R. **Media evaporation in a dry culture incubator; effect of dish, drop size and oil on media osmolality.** Fertil. Steril. 2018; 110: e363–e364

Swain, J., Kan, M., Moayeri, S., Schoolcraft, W. **Real time assessment of pH stability within an IVF isolette.** Fertil. Steril. 2015; 103: e37

Swain, J., Schoolcraft, W., Bossert, N., Batcheller, A. **Media osmolality changes over 7 days following culture in a non-humidifed benchtop incubator.** Fertil. Steril. 2016; 106: e362

Swain, J., Stevens, J., Schoolcraft, W. **Comparison of pH stability of bicarbonate bufferd media within a gassed isolette versus MOPS buffered media in room atmosphere.** Fertil. Steril. 2016; 105: e39

Swain, J. E. **Decisions for the IVF laboratory: comparative analysis of embryo culture incubators.** Reprod. Biomed. Online 2014; 28: 535–547

Swain, J. E. **Optimal human embryo culture.** Semin. Reprod. Med. 2015; 33: 103–117

Swain, J. E., Cabrera, L., Xu, X., Smith, G. D. **Microdrop preparation factors influence culture-media osmolality, which can impair mouse embryo preimplantation development.** Reprod. Biomed. Online 2012; 24: 142–147

Swain, J. E., Smith, G. D. **Advances in embryo culture platforms: novel approaches to improve preimplantation embryo development through modifications of the microenvironment.** Hum. Reprod. Update 2011; 17: 541–557

Vermilya, M., Rios, C., Abu Maizar, A., James, R., Picou, A., Werland, F., Marrs, R., Silverberg, K. **Stress Relief: can continous culture in a low lactate culture media reduce numerical chromosomal abnormaliteis and therefor improve euploidy rates.** Fertil. Steril. 2018; 110: e361–e362

Virant-Klun, I., Tomazevic, T., Vrtacnik-Bokal, E., Vogler, A., Krsnik, M., Meden-Vrtovec, H. **Increased ammonium in culture medium reduces the development of human embryos to the blastocyst stage.** Fertil. Steril. 2006; 85: 526–528

Werner, M. D., Hong, K. H., Franasiak, J. M., Forman, E. J., Reda, C. V., Molinaro, T. A., Upham, K. M., Scott, R. T.Jr. **Sequential versus Monophasic Media Impact Trial (SuMMIT): a paired randomized controlled trial comparing a sequential media system to a monophasic medium.** Fertil. Steril. 2016; 105: 1215–1221

Wolff, H. S., Fredrickson, J. R., Walker, D. L., Morbeck, D. E. **Advances in quality control: mouse embryo morphokinetics are sensitive markers of in vitro stress.** Hum. Reprod. 2013

Youssef, M. M., Mantikou, E., van Wely, M., Van der Veen, F., Al-Inany, H. G., Repping, S., Mastenbroek, S. **Culture media for human pre-implantation embryos in assisted reproductive technology cycles.** Cochrane Database Syst. Rev. 2015CD007876

Zandstra, H., Brentjens, L., Spauwen, B., Touwslager, R. N. H., Bons, J. A. P., Mulder, A. L., Smits, L. J. M., van der Hoeven, M., van Golde, R. J. T., Evers, J. L. H., Dumoulin, J. C. M., Van Montfoort, A. P. A. **Association of culture medium with growth, weight and cardiovascular development of IVF children at the age of 9 years.** Hum. Reprod. 2018; 33: 1645–1656

Zandstra, H., Van Montfoort, A. P., Dumoulin, J. C. **Does the type of culture medium used influence birthweight of children born after IVF?** Hum. Reprod. 2015; 30: 530–542

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