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Review

Paving the way for a gold standard of care for infertility treatment: improving outcomes through standardization of laboratory procedures

William Schoolcraft [a], ***Marcos Meseguer*** [b], ***The Global Fertility Alliance*** *,[1]

[a] *Colorado Center for Reproductive Medicine, Englewood, CO, USA*

[b] *Instituto Valenciano de Infertilidad (IVI) Valencia, INCLIVA-Universidad de Valencia, Valencia, Spain*

William Schoolcraft is founder and Medical Director of the Colorado Center for Reproductive Medicine, having completed his medical training at the University of Kansas and a OBGYN residency at UCLA. He pioneered blastocyst culture and transfer and has published widely on the treatment of poor responders, comprehensive chromosome screening and vitrification.

KEY MESSAGE

The best possible outcomes for IVF patients depend on optimization of many variables and procedures. A systematic approach to establishing standardized, universally-adopted best practices incorporating technological laboratory advancements should enable a gold standard of care with high-quality gametes and embryos leading to improved take-home healthy baby rates.

A B S T R A C T

Infertility affects over 70 million couples globally. Access to, and interest in, assisted reproductive technologies is growing worldwide, with more couples seeking medical intervention to conceive, in particular by IVF. Despite numerous advances in IVF techniques since its first success in 1978, almost half of the patients treated remain childless. The multifactorial nature of IVF treatment means that success is dependent on many variables. Therefore, it is important to examine how each variable can be optimized to achieve the best possible outcomes for patients. The current approach to IVF is fragmented, with various protocols in use. A systematic approach to establishing optimum best practices may improve IVF success and live birth rates. Our vision of the future is that technological advancements in the laboratory setting are standardized and universally adopted to enable a gold standard of care. Implementation of best practices for laboratory procedures will enable clinicians to generate high-quality gametes, and to produce and identify gametes and embryos of maximum viability and implantation potential, which should contribute to improving take-home healthy baby rates.

* Corresponding author.
E-mail address: alan.thornhill@igenomix.com (A Thornhill).
[1] Authors comprised Diego Ezcurra, Tammie Roy and Alan Thornhill*.

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Introduction

Assisted reproductive technologies have evolved over five decades, with several key advances, most notably the introduction of IVF (Steptoe and Edwards, 1978; Wang, 2011; Zhao et al., 2011). Despite these advances, IVF success is not guaranteed, with almost half of the patients treated remaining childless, even after multiple cycles of treatment (Centres for Disease Control and Prevention, National ART Summary Report, 2013). The live birth rates associated with IVF are surprisingly low, although they have improved slowly over time. There are wide variations in outcomes even within the same country. For example, the most recent validated and verified statistics published for IVF in the USA demonstrate a clinical pregnancy rate (per cycle started) of 21.6% at one clinic and 67% at another. Of note, these results were reported for women under 35 years of age and by clinics within 100 miles of each other (Centres for Disease Control and Prevention, Fertility Clinic Success Report, 2013). Despite the availability of new technologies and a mandatory quality system approach, results from the UK's fertility regulator (Human Fertilization and Embryology Authority [HFEA]) showed only a 1% annual increase in live birth rates after fresh embryo transfer for the reporting years 2009–11 (Human Fertilization Embryology Authority, 2013). Finally, the multiple birth rate after IVF within the developed world is vastly different, with reported rates of around 6% in Finland and Sweden, and 23% in the UK (Royal College of Obstetricians and Gynaecologists, 2011). This brief selection of examples serves to demonstrate that even with shared expertise, a vast array of literature to consult, and near global accessibility to the latest technologies and consumables, these disparities in outcomes persist and are difficult to explain as the result of purely demographic differences between patient populations. Low live birth rates can be influenced by various factors including a lack of technological proficiency at each stage of the multistep IVF process (Bhattacharya et al., 2013; Egea et al., 2014; Sunkara et al., 2014). Moreover, the financial barrier inflicted by the high cost of IVF results in a fragmented market favouring couples with sufficient financial means. Clearly the outcomes at a given centre are influenced by the patient population treated – whether that be the result of random presentation, self-selection, centre selection policies based on specific inclusion and exclusion criteria, or regulatory, legal or funding guidelines. Furthermore, it is evident that the best outcomes require appropriate and timely diagnosis of infertile patients in order to recommend and administer the appropriate and optimal ovarian stimulation and subsequent treatment. In this review, however, we focus on how technological advances in laboratory practices can address some of the challenges in IVF. Innovation in, and standardization of, laboratory practices and equipment can help optimize outcomes and improve the success rate of the current IVF treatment paradigm, paving the way for a 'gold standard' of care. To reach this standard, the benefits and limitations of existing procedures and novel technologies must be comprehensively and objectively assessed before appropriate, step-wise change can be implemented.

Subjective assessments, a major source of variability

Treatment failure can occur due to a number of factors. The complexity of the multistep IVF process results in a myriad of components that can have a detrimental influence on the outcome and directly impact live birth rates. The success of the complex process of implantation is influenced by maternal and embryonic factors, but mostly relies on cross-talk between a healthy viable embryo and a receptive endometrium (Braude, 2013). As such, generation and identification of healthy viable embryos, and evaluation of endometrial receptivity, are key. A major limitation in the characterization of endometrial receptivity and embryo implantation potential is the current lack of repeatable, easy, practical, non-invasive, cost-efficient and objective biomarkers. Currently, embryo selection is primarily based on single-point subjective morphological features, which fail to adequately discriminate between viable and non-viable embryos (with respect to both genetic and non-genetic cytoplasmic factors). This can result in the transfer of cytoplasmically or chromosomally abnormal (aneuploid) embryos that have been shown to be associated with a reduction in success (Braude, 2013; Meldrum, 2016; Meldrum and de Ziegler, 2016). Although screening tests for aneuploidy exist, the use of these tests alone or in combination with morphology varies between clinics. In addition, consensus is currently lacking regarding what type of patient may benefit from screening tests for aneuploidy, because the method is not 100% accurate and proper clinical evidence is considered by some to be insufficient (Sermon et al., 2016). Furthermore, we currently lack a reproducible test to accurately identify oocyte (hence embryo) cytoplasmic quality. Tests such as mitochondrial scoring (Diez-Juan et al., 2015; Wells et al., 2014) or oocyte viscoelastic property analysis (Yanez et al., 2016) have been suggested as a solution to this issue.

Current areas of focus for optimization of IVF laboratory techniques and procedures

There are several steps in the IVF process that could be optimized through the implementation of currently available technology. Standardizing work processes can reduce variation in IVF outcomes on both an individual and centre-wide level.

Culture media (and extended culture)

Commercialized culture media has improved IVF success and is a vital factor influencing IVF outcome, affecting pre- and post-implantation stages (Chronopoulou and Harper, 2015). Extended embryo culture prolongs growth, enables more advanced embryos to be selected, and has been linked to improved IVF success in young patients with a low body mass index (Braga et al., 2012). As with any embryo selection technique, extended culture to the blastocyst stage may shorten time to pregnancy (with higher pregnancy rates per transfer and implantation rate; Bontekoe et al., 2014; Braga et al., 2012), but does not improve cumulative pregnancy rate (De Vos et al., 2016; Glujovsky et al., 2016). Further research is required to establish optimum culture conditions for embryo development and the optimal time for transfer (Bontekoe et al., 2014).

The proliferation of commercially available culture media has seen significant improvements and optimizations in recent years and now consists of two different approaches to embryo culture: sequential, where embryos are moved part way through the culture period to a medium with a different composition; and single-step, in which embryos are held in the same dish throughout and culture medium is not replenished at any point.

In sequential culture, the different requirements of an early versus late-stage embryo are considered. For example, the energy source

provided in an early-stage medium (for embryo culture from the two pronuclear [2PN] stage until approximately day 3) is almost exclusively focused towards pyruvate and lactate, as opposed to glucose for embryo culture from day 3 onwards until embryo transfer. In addition, essential amino acids are almost completely omitted from media used for early embryo culture but are included in media used for embryos that have undergone compaction. Finally, the chelating agent ethylenediaminetetraacetic acid (EDTA) is included in media for early embryo culture but omitted from media used for extended culture, in line with the hypothesis that this compound is toxic to blastocyst stage embryos (Hardarson et al., 2015).

In single-step culture, the needs of the early (2PN stage) and late (blastocyst stage) embryos are balanced with the use of a single medium. This method is particularly advantageous when used in conjunction with continuous embryo monitoring systems, as it enables uninterrupted embryo culture and assessment from the 2PN stage until embryo transfer. This minimizes stress to embryos as a result of changes in pH, light and temperature outside of the incubator when performing static morphology grading or exposure to a medium of different composition.

Despite these different culture methods, several authors (most recently Hardarson et al., 2015) do not consider that either is 'superior' to the other. Rather, the approach selected depends on individual laboratory preferences and needs to dovetail with existing local practices. Ideally a single-step culture should be selected when using a continuous embryo monitoring system, in order to entirely benefit from uninterrupted culture and fully access the functionality of the device. With the growth in popularity of continuous embryo monitoring through time-lapse technology, one might expect to see an increase in the use of single-step versus sequential culture. Either way, the benefits of modern culture media, which are the result of multi-faceted quality control testing alongside accredited and audited manufacturing facilities, should ensure that clinics continue to purchase media from commercial sources.

Cryopreservation

Cryopreservation, which is mostly used to preserve surplus embryos and cryopreserve donated gametes or embryos for future cycles, is an integral part of today's IVF process (Zhao et al., 2011). Although useful, particularly in countries with directives to reduce multiple pregnancy rates by means of single embryo transfer, cryopreservation comes at a cost. Even the best cryopreservation protocol can generate cellular injury in embryos, which may reduce their potential to survive when compared with fresh embryos (Kopeika et al., 2015). Over the last 6–7 years, it has been demonstrated that vitrification, when performed correctly, achieves higher survival rates than slow cooling, simply by eliminating the process of crystal ice formation that can damage cellular structures and cellular membranes (Bianchi et al., 2014; Li et al., 2014). While vitrification has been shown to result in significantly higher clinical pregnancy and live birth rates when compared with slow freezing of embryos (Li et al., 2014), there are limited direct and randomized comparisons between vitrification and slow freezing in a clinical setting. However, the general trend observed in clinical practice alongside several meta-analyses (Loutradi et al., 2008; Rienzi et al., 2017) suggests that, in competent hands, vitrification has a significantly lower damage rate to eggs and embryos than slow freezing, with clinical outcomes comparable to fresh embryo transfer. The growing trend towards freeze-all cycles, separating the oocyte collection process from the embryo transfer procedure, has made the safety and efficacy of cryopreservation of paramount importance. There is accumulating evidence that this strategy provides a safer (eliminating risks of hyperstimulation) approach, with at least equivalent results (Rienzi et al., 2017) and better obstetric and perinatal outcomes versus fresh embryo transfer (Maheshwari et al., 2012).

Vitrification is much more hands-on than slow freezing, and requires a higher skill base from the technician in order to ensure the best possible outcome. Variation in outcomes between users and clinics has been reported. In order to combat this, concerted efforts need to be put into training and it has been suggested that limits should be imposed on the number of embryologists that complete the vitrification process to maintain quality (Gosden, 2011). However, this may not be necessary with additional training, benchmarking, accepted key performance indicators (Alpha Scientists in Reproductive Medicine, 2012), published standards and automation.

A number of variables have an impact on the outcome of vitrification. These include the type and concentration of cryoprotectants, temperature and timing of embryo exposure, the rate of cooling and subsequent warming, and whether or not the embryo comes into direct contact with liquid nitrogen (Gosden, 2011). As in slow freezing, where the use of a controlled-rate freezer enables the repeatable and standardized slow freezing rate that is required for successful cryopreservation, control through automation of some of these vitrification variables may further enhance repeatability and reduce the variation in outcomes.

Automation potentially provides a series of advantages over manual cryopreservation: (i) improved workflows by allowing users to process multiple embryos simultaneously, (ii) reduced training requirements and (iii) reduced intra- and inter-operator variability. Currently, there is one automated vitrification instrument, the Gavi (Genea Biomedx, Australia), commercially available in some markets, with others in development. The Gavi uses a closed system to vitrify up to four embryos simultaneously. Vitrification of mouse and human research blastocysts using automated vitrification has been shown to be equivalent in recovery, survival and development potential to that of the popular Cryotop manual vitrification method (Roy et al., 2014, 2016).

Embryo selection

Currently, most embryos are selected based upon stringent morphological factors. However, despite recent attempts to provide expert consensus regarding standardized timing and nomenclature for embryo morphological classification (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group Embryology, 2011), single-point morphology remains a subjective assessment (Braude, 2013; Wang, 2011). Morphological embryo selection is imprecise, subjective, has low predictive value for embryo implantation potential and displays relatively high inter-observer variability (even within the same centre) (Braude, 2013; Wang, 2011). Together, these findings indicate that current selection methods, employed in the vast majority of IVF cycles globally, are inadequate.

There is a strong need to develop objective biomarkers of gamete viability and embryo implantation potential that are independent of embryo appearance. Combining selection based on morphological factors with metabolic, protein and genetic markers in culture media may optimize pregnancy and live birth rates and become the standard of care by consistently selecting embryos with the highest implantation potential (Wang, 2011). When any new technology is being applied for embryo assessment, we must evaluate its efficacy by taking

into consideration the implantation potential of individual embryos by calculating the implantation rate, clinical and ongoing pregnancy rate when performing single embryo transfers, or known implantation data on patients with 0 or 100% implantation when transferring two or more embryos. In the end, the final measure of success in assisted reproductive technologies is a successful, healthy, live birth following the transfer of a single embryo (Braude, 2013; Cohen et al., 2012).

New technologies and techniques to improve embryo selection and endometrial assessment

The implementation of new technologies and techniques has the potential both to improve embryo selection, reducing reliance on subjective morphological assessment of embryo suitability, and enable more objective endometrium assessment. The development of so-called 'omics' technologies has enhanced understanding of the complex biological system surrounding reproductive success and their application should aid in reducing inter-centre variation and improve IVF outcomes generally (Egea et al., 2014).

As well as direct analysis of embryos, analysis of the cumulus cells that surround the oocyte has led to the discovery of a number of genes associated with predicting pregnancy, such as cyclooxygenase-2 and pentraxin-3 (Wang, 2011). Development of such techniques may help select the most viable embryos, leading to improved outcomes (Edgell et al., 2013; Wang, 2011). However, despite extensive efforts, reliable biomarkers for clinical application await full elucidation.

Pre-implantation genetic screening (PGS)

The most common genetic abnormality in humans is the presence of numerical chromosome abnormalities (aneuploidy) in gametes and embryos. Aneuploidy rates increase with maternal age and can be as high as 85% in women over 42 years of age, but are also present (20–25%) in young, good-prognosis patients and oocyte donors (Harton et al., 2013). Currently, no method exists to reduce the occurrence of aneuploidy in women undergoing IVF. However, the hypothesis that eliminating or deprioritizing the transfer of aneuploid embryos in favour of euploid embryos will improve implantation rates, reduce miscarriage rates, and reduce the time to pregnancy is sound, and has a growing evidence base to support it (Forman et al., 2012, 2013; Scott et al., 2013a; Yang et al., 2012). Indeed, in the absence of aneuploidy screening, embryo selection is predominantly based on morphological appearance with the risk of transferring aneuploid embryos and concomitant failed implantation, miscarriage or birth of a child with a viable trisomy (Dahdouh et al., 2015; Egea et al., 2014).

Early work using fluorescence in situ hybridization for PGS on cleavage-stage embryos at day 3 of development was shown to be ineffective and potentially harmful to a patient's chance of success (Mastenbroek et al., 2007). This was due to the reliance on optimal preparation of interphase nuclei, analysis of a limited number of chromosomes, and evaluation of cleavage-stage embryos that had a higher rate of mosaicism (potentially leading to increased risk of false-positives) and greater susceptibility to damage during biopsy (Coulam et al., 2007; Keskintepe et al., 2007). However, this landmark study clearly demonstrated that for PGS to be successful the diagnostic test would need to be highly accurate, sensitive, specific, reliable and reproducible, while the embryo biopsy would need to be as safe and effective as possible. Next-generation sequencing (NGS) solutions are predicted to overtake array comparative genomic hybridization (Orvieto et al., 2016; Simpson, 2012) as the gold standard for PGS as they appear to demonstrate improved accuracy, reproducibility and scalability, thereby reducing the cost per sample and making PGS accessible to more patients (Huang et al., 2016; Ma et al., 2016). All of these techniques use DNA extracted from embryonic cells and examine all 24 chromosomes simultaneously, which is now considered essential for optimal selection, as changes in copy numbers have been seen for all chromosomes in human IVF embryos (Franasiak et al., 2014; Huang et al., 2016; Ma et al., 2016).

PGS can be performed at different stages of embryo development by extracting DNA from the oocyte polar bodies, blastomeres at the cleavage stage, and trophectoderm from the blastocyst stage for evaluation (Dahdouh et al., 2015; Milachich, 2014). Mosaicism is the presence of two or more chromosomally different cell lineages in an embryo and occurs as a result of errors in cell division. With the trend towards more multicellular biopsy at the blastocyst stage and the emergence of sensitive NGS tools, more embryos are now being identified as exhibiting mosaicism. While this is nothing new, there is uncertainty and inconsistency as to whether embryos identified as having a single mosaic chromosome should be transferred and whether some aneuploidy and mosaicism can be attributed to the clinic and/or laboratory practice (Wells et al., 2016). While some embryos identified as mosaic (and thus classified as 'abnormal' by some clinicians and laboratories) can result in live births (Greco et al., 2015), others can result in higher rates of failed implantation or miscarriage (Maxwell et al., 2016), leading to the dilemma of whether to discard a mosaic embryo or not. Clearly the specific chromosome(s) and aneuploidy involved, combined with the degree to which mosaicism is present, are relevant factors. Recent clinical recommendations (Sachdev et al., 2017) and guidelines published by the PGD International Society provide some reassurance in this regard and recommend that a suitably accurate and sensitive test, such as NGS, should ideally be used to assess mosaicism (PGDIS, 2016).

While all of these methods are invasive, in experienced hands there is little or no detrimental effect on the developing embryo (Scott et al., 2013b). Novel non-invasive techniques for aneuploidy evaluation are being investigated. For example, differentially secreted proteins in the culture medium can be detected via proteomic technologies and mass spectrometry (McReynolds et al., 2011; Sánchez-Ribas et al., 2012). In addition, blastocoel fluid containing either cells, cell-free DNA, or both has been collected in a minimally invasive procedure to evaluate aneuploidy, with promising results in terms of concordance with the developing embryo (Geraedts et al., 2011; Milachich, 2014). Non-invasive techniques would be ideally positioned to facilitate the rapid adoption of PGS, as a major barrier in the IVF laboratory is the need for skilled biopsy practitioners. Further, the need for a non-contact laser could be reduced or completely eliminated.

Despite the failure of FISH-based PGS, recent evidence supports the use of 24-chromosome PGS to improve outcomes by deselecting aneuploid embryos (Dahdouh et al., 2015; Egea et al., 2014; Lee et al., 2015) and other larger studies are ongoing (see ClinicalTrials.gov Single Embryo TrAnsfeR of Euploid Embryo). In summary, it is fair to say that PGS continues to court controversy and there is real concern over whether embryos with an essentially 'normal' chromosomal complement are being unnecessarily discarded as a result of technical false-positives or biological mosaicism. Furthermore, successful PGS can only be achieved on a solid foundation of optimized IVF (including culture, biopsy and vitrification) using validated laboratory screening tests. As with any embryo selection,

PGS cannot increase cumulative pregnancy rates but instead may reduce time to pregnancy, financial and emotional costs of failed procedures and miscarriage. If PGS is to be routinely adopted, all component processes must be optimized to ensure that any beneficial effects seen as part of clinical trials can be transferred to all clinics worldwide.

Continuous embryo monitoring (CEM) through time-lapse technology

While genetic analysis is being proposed as a technology that can improve IVF outcomes by determining genetic status, there are other factors that need to be considered outside of the number of chromosomes present in an embryo. For an embryo to implant, it also needs to have cells with healthy cytoplasm. In some circumstances, cytoplasmic quality may be sufficient to support normal chromosome segregation but may not result in a fully competent embryo. As the embryo divides, each blastomere receives a smaller and smaller share of everything the oocyte has managed to accumulate. The fetal genome does not become capable of adding cytoplasmic factors until the cleavage stage, and the oocyte's remarkable cache of mitochondria do not start to replicate again until blastulation (Meldrum, 2016; Meldrum and de Ziegler, 2016).

Several technologies are being proposed to define the quality of the oocyte but none is yet clinically applicable. One must consider that the early embryonic divisions are a reflection of the quality of the cytoplasmic material of the oocytes, and this information can be objectively collected through CEM systems. Non-invasive embryo evaluation using CEM systems to improve pregnancy outcomes remains controversial. There are some robust data indicating an improvement over standard single-point morphological assessment (Rubio et al., 2014). However, there remains a lack of further prospective studies on the use of CEM (Kaser and Racowsky, 2015; Kovacs, 2014; Meseguer, 2016). The standard method of CEM captures images of the developing embryos for up to 7 days at 5–20 minute intervals, obtaining an unprecedented level of morphological and morphokinetic information with no interruption in culture for the embryo, and thereby maintaining the physiological environment throughout the culture period (Basile et al., 2015; Kovacs, 2014). Such systems provide a method of embryo assessment that diminishes observer subjectivity, reveals hitherto unseen processes and anomalies, and limits exposure to non-physiological conditions. Standard static morphological assessment requires embryologists to remove embryos from the incubator to visually assess cleavage and morphology at single points in time over the course of several days, which disturbs the culture conditions and can impact embryo development (Park et al., 2015). These snapshots may well represent false readings, as embryonic development is a dynamic process and key events may be missed if they occur between observations (Aparicio et al., 2013). Together these factors limit the value of current static morphological assessment. CEM may provide the solution to this significant problem as 24-hour monitoring can be achieved without removing embryos from the culture medium. Several types of equipment exist, some including high-definition cameras and the incubation system (Geri, EmbryoScope, Miri TL), others consisting only of cameras that can be included inside box incubators (Embryo Evaluation Viability Assessment [Eeva], Primo-Vision). The Eeva system differs from other available systems as it uses a proprietary validated algorithm that automatically predicts blastocyst development based on timings of specific events during the first few cleavage divisions, and has been shown to be more effective in this regard than experienced embryology observers alone (Conaghan et al., 2013). Conflicting results on the impact of undisturbed embryo culture have been reported, but a 1-year retrospective study indicated that undisturbed embryo culture in the EmbryoScope incubator may improve live birth rates, and that conventional culture methods may negatively impact embryo growth and implantation potential (McEvoy et al., 2016). Several randomized controlled trials are under way to evaluate the clinical value of having an objective CEM system and uninterrupted culture conditions for early embryonic stages. Nevertheless, current retrospective and prospective studies underline the utility of this technology in a clinical setting with promising results (Adamson et al., 2015; Diamond et al., 2015; VerMilyea et al., 2014). CEM has been adopted by many clinics and several studies have been completed demonstrating the incremental utility of combining innovative technologies such as PGS with CEM, to objectively identify genetically and cytoplasmically healthy day 3 and day 5 embryos (Dominguez et al., 2015; Rocafort et al., 2016; Rubio et al., 2014; Yang et al., 2014). As with so many technological advances, CEM may not yield immediate benefits to all laboratories and successful implementation probably requires some standardization (e.g. with respect to embryo stage annotation). Indeed, CEM has not universally shown embryo selection benefits in clinical trials (Goodman et al., 2016) and it can be difficult to tease apart potential clinical benefits from an uninterrupted culture system from those obtained via improved embryo selection. However, its additional value in embryo deselection (Athayde Wirka et al., 2014; Liu et al., 2016), facilitating uninterrupted culture, as a sensitive quality control tool (Wolff et al., 2013) and an aid to appropriate timing of procedures in the embryology laboratory (e.g. embryo biopsy) are not without incremental value. Additional large, robust clinical trials will help to guide the future utility of this exciting technology (Kaser and Racowsky, 2015).

Assessing receptivity of the endometrium

Cross-talk between the transferred embryo and the endometrium is critical for implantation success (Edgell et al., 2013). Endometrial receptivity is limited to 4 days during the mid-secretory phase of the menstrual cycle (Egea et al., 2014). As the window of implantation is small, assessing endometrial receptivity is vital in order to establish the optimum time for embryo transfer (Edgell et al., 2013). The current method is to evaluate receptivity through assessment of morphology-based histological features. However, this technique is questionable as the endometrial tissue is highly dynamic and changes according to menstrual phase, and with repeated implantation and stimulation (Edgell et al., 2013; Egea et al., 2014). Initiatives to develop reproducible methods for determining endometrial receptivity are ongoing. These include the investigation into the use of lipids, mRNA, genes, peptides and cytokines (Boosma et al., 2009; Casado-Vela et al., 2009; Chan et al., 2013; Edgell et al., 2013; Haouzi et al., 2009; Simon and Laufer, 2012).

Conclusion

Advances in IVF have already led to improvements in key aspects of the processes involved, improving success rates and making IVF safer. Nevertheless, further enhancements can be made by taking a compartmentalized view of the process and implementing currently

available validated technologies such as PGS, CEM and automated vitrification where possible, practical and clinically indicated. These incremental advances may quickly accumulate, providing real improvement in take-home healthy baby rates.

We have highlighted several specific technologies associated with embryo selection and embryo vitrification but as the power of automation, DNA sequencing and other innovative and disruptive technologies fully penetrate the fertility arena we expect to see many more processes refined. The laboratory is an excellent model for such incremental improvements as the processes, environment and equipment used are controlled and measured frequently and with precision. The vision for the future of IVF is that each and every step in the process is optimized, refined and implemented to the highest possible gold standard. Applying the philosophy of incremental gains will be gradual and, where possible, requires both existing and developing methodologies to be optimized and validated in large retrospective or prospective randomized studies with the support of the global infertility community. Our hope is that evaluation of a validated, standardized approach will help achieve the ultimate goal of IVF treatment, which is to achieve a healthy, single, live birth from the transfer of a single embryo. The first steps towards this goal would be to develop best practice guidelines, key performance indicators and benchmarks for all aspects of the IVF process, as have been established for cryopreservation (Alpha Scientists in Reproductive Medicine, 2012). Moreover, the IVF community and its patients could benefit from an integrated database or data-warehousing system that leverages, in real time, the power of thousands or even millions of data points captured from routine, advanced and technology-driven processes generated daily across different centres. Finally, a willingness from the IVF community to accept that current standards are frequently both inadequate and outdated would help to initiate change. By introducing global quality standards and benchmarking, and by embracing new technologies, providers could rapidly assess their value and implement them within their centres.

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REFERENCES

Adamson, G.D., Abusief, M.E., Palao, L.M., Gvakharia, M., 2015. Improved implantation rates of day 3 embryo transfers with the use of an automated time-lapse–enabled test to aid in embryo selection. Fertil. Steril. 105, 369–375.

Alpha Scientists in Reproductive Medicine, 2012. The Alpha consensus meeting on cryopreservation key performance indicators and benchmarks: proceedings of an expert meeting. Reprod. Biomed. Online 25, 146–167.

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group Embryology, 2011. Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Reprod. Biomed. Online 22, 632–646.

Aparicio, B., Cruz, M., Meseguer, M., 2013. Is morphokinetic analysis the answer? Reprod. Biomed. Online 27, 654–663.

Athayde Wirka, K., Chen, A.A., Conaghan, J., Ivani, K., Gvakharia, M., Behr, B., Suraj, V., Tan, L., Shen, S., 2014. Atypical embryo phenotypes identified by time-lapse microscopy: high prevalence and association with embryo development. Fertil. Steril. 101, 1637–1648.

Basile, N., Caiazzo, M., Meseguer, M., 2015. What does morphokinetics add to embryo selection and in-vitro fertilization outcomes? Curr. Opin. Obstet. Gynecol. 27, 193–200.

Bhattacharya, S., Maheshwari, A., Mollison, J., 2013. Factors associated with failed treatment: an analysis of 121,744 women embarking on their first IVF cycles. PLoS ONE 8, 2249.

Bianchi, V., Macchiarelli, G., Borini, A., Lappi, M., Cecconi, S., Miglietta, S., Familiari, G., Nottola, S.A., 2014. Fine morphological assessment of quality of human mature oocytes after slow freezing or vitrification with a device: a comparative analysis. Reprod. Biol. Endocrinol. 12, 110.

Bontekoe, S., Heineman, M.J., Johnson, N., Blake, D., 2014. Adherence compounds in embryo transfer media for assisted reproductive technologies. Cochrane Database Syst. Rev. (2), CD007421.

Boosma, C.M., Kavelaars, A., Eijkemans, M.J., Lentjes, E.G., Fauser, B.C., Heijnen, C.J., Macklon, N.S., 2009. Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. Hum. Reprod. 24, 1427–1435.

Braga, D.P., Setti, A.S., de Cássia, S., Figueira, R., Machado, R.B., Iaconelli, A., Jr., Borges, E., Jr., 2012. Patient selection criteria for blastocyst transfers in extended embryo culture programs. J. Assist. Reprod. Genet. 29, 1357–1362.

Braude, P., 2013. Selecting the 'best' embryos: prospects for improvement. Reprod. Biomed. Online 27, 644–653.

Casado-Vela, J., Rodriquez-Suarez, E., Iloro, I., Ametzazurra, A., Alkorta, N., García-Velasco, J.A., Matorras, R., Prieto, B., González, S., Nagore, D., Simón, L., Elortza, F., 2009. Comprehensive proteomic analysis of human endometrial fluid aspirate. J. Proteome Res. 8, 4622–4632.

Centres for Disease Control and Prevention. Assisted reproductive technology: ART trends 2002–2011. http://www.cdc.gov/art/ART2011/section5.htm#f43. (Accessed 16 August 2016).

Centres for Disease Control and Prevention. Assisted Reproductive Technology Fertility Clinic Success Rates Report. http://www.cdc.gov/art/pdf/2013-report/art-2013-fertility-clinic-report.pdf. (Accessed 16 August 2016).

Centres for Disease Control and Prevention. Assisted Reproductive Technology National Summary Report 2013. http://www.cdc.gov/art/pdf/2013-report/art_2013_national_summary_report.pdf. (Accessed 16 August 2016).

Chan, C., Virtanen, C., Winegarden, N.A., Colgan, T.J., Greenblatt, E.M., 2013. Discovery of biomarkers of endometrial receptivity through a minimally invasive approach: a validation study with implications for assisted reproduction. Fertil. Steril. 100, 810–817.

Chronopoulou, E., Harper, J.C., 2015. IVF culture media: past present and future. Hum. Reprod. Update 21, 39–55.

ClinicalTrials.gov Single Embryo TrAnsfeR of Euploid Embryo (STAR). NCT02268786. https://clinicaltrials.gov/ct2/show/NCT02268786. (Accessed 26 April 2016).

Cohen, J., Alikani, M., Bisignano, A., 2012. Past performance of assisted reproduction technologies as a model to predict future progress: a proposed addendum to Moore's law. Reprod. Biomed. Online 25, 585–590.

Conaghan, J., Chen, A.A., Willman, S.P., Ivani, K., Chenette, P.E., Boostanfar, R., Baker, V.L., Adamson, G.D., Abusief, M.E., Gvakharia, M., Loewke, K.E., Shen, S., 2013. Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: results from a prospective multicentre trial. Fertil. Steril. 100, 412–419.

Coulam, C.B., Jeyendran, R.S., Fiddler, M., Pergament, E., 2007. Discordance among blastomeres renders preimplantation genetic diagnosis for aneuploidy ineffective. J. Assist. Reprod. Genet. 24, 37–41.

Dahdouh, E.M., Balayla, J., Audibert, F., Genetics Committee, Wilson, R.D., Audibert, F., Brock, J.A., Campagnolo, C., Carroll, J., Chong, K., Gagnon, A., Johnson, J.A., MacDonald, W., Okun, N., Pastuck, M., Vallée-Pouliot, K., 2015. Technical update: preimplantation genetic diagnosis and screening. J. Obstet. Gynaecol. Can. 37, 451–463.

De Vos, A., Van Landuyt, L., Santos-Ribeiro, S., Camus, M., Van de Velde, H., Tournaye, H., Verheyen, G., 2016. Cumulative live birth rates after fresh and vitrified cleavage-stage versus blastocyst-stage embryo transfer in the first treatment cycle. Hum. Reprod. 31, 2442–2449.

Diamond, M.P., Suraj, V., Behnke, E.J., Yang, X., Angle, M.J., Lambe-Steinmiller, J.C., Watterson, R., Athayde Wirka, K., Chen, A.A., Shen, S., 2015. Using the Eeva Test™ adjunctively to traditional day 3 morphology is informative for consistent embryo assessment within a panel of embryologists with diverse experience. J. Assist. Reprod. Genet. 32, 61–68.

Diez-Juan, A., Rubio, C., Marin, C., Martinez, S., Al-Asmar, N., Riboldi, M., Díaz-Gimeno, P., Valbuena, D., Simón, C., 2015. Mitochondrial DNA content as a viability score in human euploid embryos: less is better. Fertil. Steril. 104, 534–541.

Dominguez, F., Meseguer, M., Aparicio-Ruiz, B., Piqueras, P., Quiñonero, A., Simón, C., 2015. New strategy for diagnosing embryo implantation potential by combining proteomics and time-lapse technologies. Fertil. Steril. 104, 908–914.

Edgell, T.A., Rombauts, L.J., Salamonsen, L.A., 2013. Assessing receptivity in the endometrium: the need for a rapid, non-invasive test. Reprod. Biomed. Online 27, 486–496.

Egea, R.R., Puchalt, N.G., Escrivá, M.M., Varghese, A.C., 2014. Current and future perspectives in reproductive medicine and technology. J. Hum. Reprod. Sci. 7, 73–92.

Forman, E.J., Tao, X., Ferry, K.M., Taylor, D., Treff, N.R., Scott, R.T., Jr., 2012. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Hum. Reprod. 27, 1217–1222.

Forman, E.J., Hong, K.H., Ferry, K.M., Tao, X., Taylor, D., Levy, B., Treff, N.R., Scott, R.T., Jr., 2013. *In vitro* fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil. Steril. 100, 100–107.

Franasiak, J.M., Forman, E.J., Hong, K.H., Werner, M.D., Upham, K.M., Treff, N.R., Scott, R.T., 2014. Aneuploidy across individual chromosomes at the embryonic level in trophectoderm biopsies: changes with patient age and chromosome structure. J. Assist. Reprod. Genet. 31, 1501–1509.

Geraedts, J., Montag, M., Magli, M.C., Repping, S., Handyside, A., Staessen, C., Harper, J., Schmutzler, A., Collins, J., Gossens, V., van der Ven, H., Vesela, K., Gianaroli, L., 2011. Polar body array CGH for prediction of the status of the corresponding oocyte. PART I: clinical results. Hum. Reprod. 26, 3173–3180.

Glujovsky, D., Farquhar, C., Quinteiro Retamar, A.M., Alvarez Sedo, C.R., Blake, D., 2016. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst. Rev. (6), CD002118.

Goodman, L.R., Goldberg, J., Falcone, T., Austin, C., Desai, N., 2016. Does the addition of time-lapse morphokinetics in the selection of embryos for transfer improve pregnancy rates? A randomized controlled trial. Fertil. Steril. 105, 275–285.

Gosden, R., 2011. Cryopreservation: a cold look at technology for fertility preservation. Fertil. Steril. 96, 264–268.

Greco, E., Minasi, M., Fiorentino, F., 2015. Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts. N. Engl. J. Med. 373, 2089–2090.

Haouzi, D., Assou, S., Mahmoud, K., Tondeur, S., Réme, T., Hedon, B., De Vos, J., Hamamah, S., 2009. Gene expression profile of human endometrial receptivity: comparison between natural and stimulated cycles for the same patients. Hum. Reprod. 24, 1436–1445.

Hardarson, T., Bungum, M., Conaghan, J., Meintjes, M., Chantilis, S.J., Molnar, L., Gunnarsson, K., Wikland, M., 2015. Noninferiority, randomized, controlled trial comparing embryo development using media developed for sequential or undisturbed culture in a time-lapse setup. Fertil. Steril. 104, 1452–1459.

Harton, G.L., Munné, S., Surrey, M., Grifo, J., Kaplan, B., McCulloh, D.H., Griffin, D.K., Wells, D., 2013. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. Fertil. Steril. 100, 1695–1703.

Huang, J., Yan, L., Lu, S., Zhao, N., Xie, X.S., Qiao, J., 2016. Validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of blastocysts. Fertil. Steril. 105, 1532–1536.

Human Fertilization Embryology Authority. Fertility treatment in 2013, trends and figures 2013. http://ifqtesting.blob.core.windows.net/umbraco-website/1142/hfea_fertility_trends_and_figures_2013.pdf. (Accessed 28 April 2016).

Kaser, D.J., Racowsky, C., 2015. Reply: clinical outcomes following selection of human preimplantation embryos with time-lapse monitoring: a systematic review. Hum. Reprod. Update 20, 617–631.

Keskintepe, L., Sher, G., Keskintepe, M., 2007. Reproductive oocyte/embryo genetic analysis: comparison between fluorescence in-situ hybridization and comparative genomic hybridization. Reprod. Biomed. Online 15, 303–309.

Kopeika, J., Thornhill, A., Khalaf, Y., 2015. The effect of cryopreservation on the genome of gametes and embryos: principles of cryobiology and critical appraisal of the evidence. Hum. Reprod. Update 21, 209–227.

Kovacs, P., 2014. Embryo selection: the role of time-lapse monitoring. Reprod. Biol. Endocrinol. 12, 124.

Lee, E., Illingworth, P., Wilton, L., Chambers, G.M., 2015. The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review. Hum. Reprod. 30, 473–483.

Li, Z., Wang, Y.A., Ledger, W., Edgar, D.H., Sullivan, E.A., 2014. Clinical outcomes following cryopreservation of blastocysts by vitrification or slow freezing: a population-based cohort study. Hum. Reprod. 29, 2794–2801.

Liu, Y., Chapple, V., Feenan, K., Roberts, P., Matson, P., 2016. Time-lapse deselection model for human day 3 *in vitro* fertilization embryos: the combination of qualitative and quantitative measures of embryo growth. Fertil. Steril. 90, 186–193.

Loutradi, K.E., Kolibianakis, E.M., Venetis, C.A., Papanikolaou, E.G., Pados, G., Bontis, I., Tarlatzis, B.C., 2008. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. Fertil. Steril. 90, 186–193.

Ma, G.C., Chen, H.F., Yang, Y.S., Lin, W.H., Tsai, F.P., Lin, C.F., Chiu, C., Chen, M., 2016. A pilot proof-of-principle study to compare fresh and vitrified cycle preimplantation genetic screening by chromosome microarray and next generation sequencing. Mol. Cytogenet. 9, 25.

Maheshwari, A., Kalampokas, T., Davidson, J., Bhattacharya, S., 2012. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of blastocyst-stage versus cleavage-stage embryos generated through *in vitro* fertilization treatment: a systematic review and meta-analysis. Fertil. Steril. 98, 368–377.

Mastenbroek, S., Twisk, M., van Echten-Arendts, J., Sikkema-Raddatz, B., Korevaar, J.C., Verhoeve, H.R., Vogel, N.E., Arts, E.G., de Vries, J.W., Bossuyt, P.M., Buys, C.H., Heineman, M.J., Repping, S., van der Veen, F., 2007. *In vitro* fertilization with preimplantation genetic screening. N. Engl. J. Med. 357, 9–17.

Maxwell, S., Colls, P., Hodes-Wertz, B., McCulloh, D.H., McCaffrey, C., Wells, D., Munné, S., Grifo, J.A., 2016. Why do euploid embryos miscarry? A case-control study comparing the rate of aneuploidy within presumed euploid embryos that resulted in miscarriage or live birth using next-generation sequencing. Fertil. Steril. 106, 1414–1419.

McEvoy, K., Brison, D., Roberts, S., 2016. A one year retrospective analysis comparing live birth outcomes from embryos grown and transferred from an undisturbed time-lapse culture system with a conventional culture system. Presented at the 32nd Annual Meeting of the European Society of Human Reproduction and Embryuology in Helsinki, Finland, 3–6 July 2016. Abstract P-106.

McReynolds, S., Vanderlinden, L., Stevens, J., Hansen, K., Schoolcraft, W.B., Katz-Jaffe, M.G., 2011. Lipocalin-1: a potential marker for noninvasive aneuploidy screening. Fertil. Steril. 95, 2631–2633.

Meldrum, D.R., 2016. Introduction: examining the many potential reasons why euploid blastocysts do not always result in viable pregnancies: part 1. Fertil. Steril. 105, 545–547.

Meldrum, D.R., de Ziegler, D., 2016. Introduction: examining the many potential reasons why euploid blastocysts do not always result in viable pregnancies (and deliveries): part 2. Fertil. Steril. 105, 841–843.

Meseguer, M., 2016. Time-lapse: the remaining questions to be answered. Fertil. Steril. 105, 295–296.

Milachich, T., 2014. New advances of preimplantation and prenatal genetic screening and noninvasive testing as a potential predictor of health status of babies. Biomed Res. Int. 2014, 1–8.

Orvieto, R., Shuly, Y., Brengauz, M., 2016. Should pre-implantation genetic screening be implemented to routine clinical practice? Gynecol. Endocrinol. 12, 1–3.

Park, H., Bergh, C., Selleskog, U., Thurin-Kjelberg, A., Lundin, K., 2015. No benefit of culturing embryos in a closed system compared with a conventional incubator in terms of number of good quality embryos: results from an RCT. Hum. Reprod. 30, 268–275.

PGDIS, 2016. Position statement on chromosome mosaicism and preimplantation aneuploidy testing at the blastocyst stage. PGDIS Newsletter, July 19, 2016. (see http://www.pgdis.org/docs/newsletter_071816.html).

Rienzi, L., Gracia, C., Maggiulli, R., LaBarbera, A.R., Kaser, D.J., Ubaldi, F.M., Vanderpoel, S., Racowsky, C., 2017. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum. Reprod. Update 23, 139–155.

Rocafort, E., Leza, A., Ramos, B., 2016. Combined time-lapse imaging and preimplantation genetic screening: a valuable strategy for embryo selection. Presented at the 32nd Annual Meeting of the European Society of Human Reproduction and Embryology in Helsinki, Finland, 3–6 July 2016. Abstract P-642.

Roy, T.K., Brandi, S., Tappe, N.M., Bradley, C.K., Vom, E., Henderson, C., Lewis, C., Battista, K., Hobbs, B., Hobbs, S., Syer, J., Lanyon, S.R., Dopheide, S.M., Peura, T.T., McArthur, S.J., Bowman, M.C., Stojanov, T., 2014. Embryo vitrification using a novel semi-automated closed system yields *in vitro* outcomes equivalent to manual Cryotop method. Hum. Reprod. 29, 2431–2438.

Roy, T.K., Brandi, S., Bradley, C.K., 2016. Automatic vitrification: development of the Gavi system. In: Tucker, M.J., Liebermann, J. (Eds.), Vitrification in Assisted Reproduction, second ed. CRC Press, Florida, pp. 61–69.

Royal College of Obstetricians and Gynaecologists. 2011. Multiple Pregnancy Following Assisted Reproduction. https://www.rcog.org.uk/globalassets/documents/guidelines/sip_no_22.pdf. (Accessed 26 April 2016).

Rubio, I., Galán, A., Larreategui, Z., Ayerdi, F., Bellver, J., Herrero, J., Meseguer, M., 2014. Clinical validation of embryo culture and selection by morphokinetic analysis: a randomized, controlled trial of the EmbryoScope. Fertil. Steril. 102, 1287–1294.

Sánchez-Ribas, J., Riqueros, M., Vime, P., Puchades-Carrasco, L., Jönsson, T., Pineda-Lucena, A., Ballesteros, A., Domínguez, F., Simón, C., 2012. Differential metabolic profiling of non-pure trisomy 21 human preimplantation embryos. Fertil. Steril. 98, 1157–1164.

Sachdev, N.M., Maxwell, S.M., Besser, A.G., Grifo, A.J., 2017. Diagnosis and clinical management of embryonic mosaicism. Fertil. Steril. 107, 6–11.

Scott, R.T., Upham, K.M., Forman, E.F., Hong, K.H., Scott, K.L., Taylor, D., Tao, X., Treff, N.R., 2013b. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases IVF IRs and delivery rates: a randomized controlled trial. Fertil. Steril. 100, 697–703.

Scott, R.T., Jr., Upham, K.M., Forman, E.J., Zhao, T., Treff, N.R., 2013a. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil. Steril. 100, 624–630.

Sermon, K., Capalbo, A., Cohen, J., Coonen, E., De Rycke, M., De Vos, A., Delhanty, J., Fiorentino, F., Gleicher, N., Griesinger, G., Grifo, J., Handyside, A., Harper, J., Kokkali, G., Mastenbroek, S., Meldrum, D., Meseguer, M., Montag, M., Munné, S., Rienzi, L., Rubio, C., Scott, K., Scott, R., Simon, C., Swain, J., Treff, N., Ubaldi, F., Vassena, R., Vermeesch, J.R., Verpoest, W., Wells, D., Geraedts, J., 2016. The why, the how and the when of PGS 2.0: current practices and expert opinions of fertility specialists, molecular biologists, and embryologists. Mol. Hum. Reprod. 22, 845–857.

Simon, A., Laufer, N., 2012. Assessment and treatment of repeated implantation failure (RIF). J. Assist. Reprod. Genet. 29, 1227–1239.

Simpson, J.L., 2012. Preimplantation genetic diagnosis to improve pregnancy outcomes in subfertility. Best Pract. Res. Clin. Obstet. Gynaecol. 26, 805–815.

Steptoe, P., Edwards, R.G., 1978. Birth after the reimplantation of a human embryo. Lancet 2, 366.

Sunkara, S.K., Khalaf, Y., Maheschwari, A., Seed, P., Coomarasamy, A., 2014. Association between response to ovarian stimulation and miscarriage following IVF: an analysis of 124 351 IVF pregnancies. Hum. Reprod. 29, 1218–1224.

VerMilyea, M.D., Tan, L., Anthony, J.T., Conaghan, J., Ivani, K., Gvakharia, M., Boostanfar, R., Baker, V.L., Suraj, V., Chen, A.A., Mainigi, M., Coutifaris, C., Shen, S., 2014. Computer-automated time-lapse analysis results correlate with embryo implantation and clinical pregnancy: a blinded, multi-centre study. Reprod. Biomed. Online 29, 729–736.

Wang, S.X., 2011. The past, present and future of embryo selection in *in vitro* fertilization: Frontiers in Reproductive Conference. Yale J. Biol. Med. 84, 487–490.

Wells, D., Kaur, K., Grifo, J., Glassner, M.J., Taylor, J.C., Fragouli, E., Munne, S., 2014. Clinical utilisation of a rapid low-pass whole genome sequencing technique for the diagnosis of aneuploidy in human embryos prior to implantation. J. Med. Genet. 51, 553–562.

Wells, D., Alfarawati, S., Taylor, S., 2016. Evidence that differences between embryology laboratories can influence the rate of mitotic errors, leading to increased chromosomal mosaicism, with significant implications for IVF success rates. Presented at the 32nd Annual Meeting of the European Society of Human Reproduction and Embryuology in Helsinki, Finland, 3–6 July 2016. Abstract O-058.

Wolff, H., Fredrickson, J., Walker, D., Morbeck, D.E., 2013. Advances in quality control: mouse embryo morphokinetics are sensitive markers of *in vitro* stress. Hum. Reprod. 28, 1776–1782.

Yanez, L.Z., Han, J., Behr, B.B., Reijo Pera, R.A., Camarillo, D.B., 2016. Human oocyte developmental potential is predicted by mechanical properties within hours after fertilization. Nat. Commun. 7, 10809.

Yang, Z., Liu, J.J., Collins, G.S., Salem, S.A., Liu, X., Lyle, S.S., Peck, A.C., Sills, E.S., Salem, R.D., 2012. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol. Cytogenet. 5, 24.

Yang, Z., Zhang, J., Salem, S.A., Liu, X., Kuang, Y., Salem, R.D., Liu, J., 2014. Selection of competent blastocysts for transfer by combining time-lapse monitoring and array CGH testing for patients undergoing preimplantation genetic screening: a prospective study with sibling oocytes. BMC Med. Genomics 7, 38.

Zhao, Y., Brezina, P., Hsu, C.C., Garcia, J., Brinsden, P.R., Wallach, E., 2011. *In vitro* fertilization: four decades of reflections and promises. Biochim. Biophys. Acta 1810, 843–852.