RBMO

COUNTERCURRENT

'The way to improve ART outcomes is to introduce more technologies in the laboratory'

David K. Gardner*

ABSTRACT

To address the proposition that 'the way to improve ART outcomes is through the introduction of more technologies in the laboratory', it is prudent to first define what is considered to be improved outcomes. Evidently, this equates to an increase in the live birth rate but it should also include parameters such as time to pregnancy, cumulative pregnancy per oocyte retrieval and health of the resultant child. Furthermore, being able to maintain clinical results week in, week out through quality management also contributes to the overall success of a clinic, and hence can be considered an improved outcome. With regards to these outcomes, it is offered that not only does the introduction of several new technologies (defined here as instrumentation, techniques and enhanced computer utilization and analysis) have the potential to improve outcomes, but also some of them have the capacity to facilitate automation and standardization in the ART laboratory. Although the automation of procedures can be perceived as a justifiable goal itself, in this contribution the emphasis is on how new technologies could help more patients become parents of healthy children in the shortest possible time.

IN THE BEGINNING

At the time Louise Brown was born in 1978, there was limited technology to help undertake the task of human IVF, and both scientists and clinicians had to be creative with equipment procured from other fields of biomedical research and clinical practice. In many ways the initial days of IVF depended heavily on both intellect and hand skills to literally craft ways to maintain the embryo in a 'laboratory environment', which provided a less than favourable environment for the process of IVF; for example, it was not uncommon to find the assisted reproductive technology (ART) laboratory in a converted janitor's closet close to the retrieval room. When I started out on my own journey into IVF, some 40 years ago, desktop computers were a thing of the future and PhD theses were handwritten and subsequently typed. There were no ready-made media, or the capacity for multigas incubation, save for using some form of modular chamber such as a glass desiccator that could be purged with pre-mixed gasses (the so-called 'Womb with a View'). To make matters more challenging, every mammalian species from the mouse to the sheep, to the cow and the human had a developmental block in culture and viable blastocyst development could not readily be attained.

Therefore, for no other reason than necessity, we resorted to transferring embryos to the uterus as soon as possible, at the cleavage stages (which in both laboratory and domestic species is known to compromise transfer outcome). Hence this approach culminated in two issues: (i) low pregnancy rates resulting in multiple cycle attempts, and (ii) a paradoxical increase in multiple gestations associated with infertility, due to the transfer of several embryos in the hope of increasing pregnancy rates.

Melbourne IVF and School of BioSciences, University of Melbourne, Australia

*Corresponding author. E-mail address: david.gardner@unimelb.edu.au
https://doi.org/10.1016/j.rbmo.2021.10.021 1472-6483/
Declaration: The authors reports no financial or commercial conflicts of interest.

KEYWORDS
Artificial Intelligence
Biomarkers
IVF
Microfabrication
Microfluidics
Microperfusion

Hence, by the criteria set out above, human IVF had poor outcomes.

THE EMBRYOLOGY RENAISSANCE

Fortuitously, in the intervening decades extensive research on mammalian embryo physiology and metabolism, together with the analysis of human oviduct and uterine fluids, led to the formulation of human embryo-specific media systems, providing the ability to culture to the viable blastocyst stage (*Gardner and Lane, 2003*). Furthermore, male factor infertility was largely overcome through the introduction of intracytoplasmic sperm injection (ICSI). Concomitantly, research on vitrification culminated in improved cryopreservation systems for gametes and embryos (*Nagy et al., 2020*).

This time also witnessed the development of equipment specifically for human IVF, such as embryo workstations (originally derived from paediatric isolettes) to maintain a constant environment for gamete and embryo manipulation, and the introduction of appropriate laboratory design and building standards, combined with air filtration to reduce both particulates and volatile organic compounds in laboratories (*Esteves and Bento, 2016*). In parallel, more stringent quality control and management systems were implemented. As a result, we have witnessed an increase in pregnancy rates, a reduction in the number of embryos transferred (today single blastocyst transfer is the standard of care) and an increase in cumulative pregnancy rates.

ART LABORATORY OF THE 20TH CENTURY

So, what contributed to the increase in ART success, the basic science (such as that which underpinned the development of better culture conditions) or technological development? Clearly, they are not independent, as more detailed scientific investigation is facilitated by better technologies, but do we really need more technology in the clinic? It is evident that a skilled experienced embryologist working together with an expert IVF physician can produce excellent clinical pregnancy rates, even when using basic technologies. Such an embryologist provides insight into embryo manipulation and culture, knowing exactly how to handle the embryo (with slow movement and minimal volumes of media), possessing a deftness of touch during ICSI, and having an excellent eye for embryo grading, being both accurate and reproducible in their blastocyst selection.

However, while such a scenario works well with very low case numbers (of just a few hundred cycles per year), we live and work in a world where there is an ever-increasing demand for ART and where clinics are getting larger, with the projected growth of human IVF over the next decade being considerable. This latter point raises the issue of where are all the highly trained embryologists and physicians required for this growth going to come from, and how can consistency of treatment results be obtained? Furthermore, as clinics increase in size how can outcomes be standardized, for example when embryo assessment is notoriously subjective?

ART LABORATORY OF THE 21ST CENTURY

It is envisaged that technologies have an ever-increasing role to play, not only to help improve outcomes, but also to help standardize practice. In 1994 I wrote a paper on the future of embryo culture that projected great hope and promise, such as the introduction of blastocyst transfer, trophectoderm biopsy, the assessment of viability through biomarkers, and the introduction of perfusion culture, while also highlighting the constraints and compromises faced when trying to maintain an embryo *in vitro* (*Gardner, 1994*). Although much of this is now considered routine practice, it is still not possible to utilize the potential benefits of perfusion culture (which could provide gradients of nutrients and removal of waste products in real time), because the technologies required are not yet fully realized. However, this is now rapidly changing, and it will not be long before such a dynamic approach to routine embryo culture can be evaluated clinically.

We have already observed the transformation of genetic analysis of embryos as techniques have moved from day 3 to blastocyst biopsy, and from fluorescence in-situ hybridization to next-generation sequencing, with the potential to reduce time to pregnancy and the incidence of miscarriage in women aged over 35 years (*Munne et al., 2019*). The introduction of electronic witnessing has helped to remove human errors in the laboratory, thereby also contributing to success, and there has been a development of time-lapse microscopy systems specifically for embryo culture and analysis. Although there are those who do not see this as a major advance, others see it as a means not only for standardization of embryo analysis (especially when linked with algorithms or artificial intelligence [AI]), but also as a valuable tool in quality management of the laboratory. But what of the future? TABLE 1 highlights several novel technologies, not yet introduced clinically, all of which have the potential to further increase ART outcomes, as defined above.

With advances in microfabrication and lab-on-a-chip technologies (including microfluidics), the reality of dynamic culture will be realized, as will both the selection and diagnosis of gametes. Microrobotics are being developed to remove human errors associated with ICSI (*Lu et al., 2011*), while novel microscopies such as hyperspectral and fluorescence lifetime imaging (FLIM) are being developed to not only assess the metabolic state of the embryo, but also indirectly give a measure of the epigenetic state (*Gardner et al., 2019*). Advances in biomarker discovery and enhanced DNA analysis could further see the introduction of a means to assess embryo health completely non-invasively through the analysis of spent culture media.

Furthermore, many of the technologies in TABLE 1 are aligned with automation, thereby paving the way for the next generation of IVF laboratories, which will increasingly benefit from utilizing AI. There has been an initial introduction of AI in the IVF clinic, where it is currently been evaluated to assist in sperm selection, in embryo selection for transfer (*Tran et al., 2019*), in the quality management and performance of our laboratories (*Bormann, et al., 2021*) and in helping to optimize stimulation protocols. The use of AI will continue to expand as an increasing amount of data on embryos is created, facilitated through the technologies considered in TABLE 1; this will help to create new algorithms of 'embryo health' made feasible by the collection of data on the genetic, epigenetic and physiological state of the embryo, thereby improving outcomes (*Ferrick et al., 2019*).

TABLE 1 POTENTIAL IMPACT OF POTENTIAL NEW TECHNOLOGIES ON ART OUTCOMES

Technology	Description/role	Manual or automated	Impact on outcome
Microperfusion	Capacity to change the environment around the gametes/embryo in real time and create stage-specific environments	Automated	Less stress associated with medium renewal will lead to increased embryo development and viability, and hence increasing pregnancy rates
	Capacity to introduce cryoprotectants as gradual gradients rather than fixed steps	Automated	Will increase the cryo-survival of gametes and embryos and hence increase cumulative pregnancy rates
Microfluidics	Ability to sort and select viable sperm	Automated	Increased fertilization rates, resulting in more embryos per cycle and reduced pregnancy losses
Microfabrication	3D printing to fabricate embryo chambers, thereby facilitating the creation of microenvironments to support embryo development and which are compatible with microperfusion culture technology	Automated	Improved embryo development and pregnancy rates
Microrobotics	Ability to perform and automate procedures such as ICSI (potentially incorporating piezo technology), which require micromanipulation	Automated	Increased consistency of fertilization, culminating in more, and potentially better quality, embryos
Time release of embryo trophic factors	Particle-based system for time- and stage-specific delivery of factors to the culture medium	Manual	Increased embryo development and pregnancy rates
Novel microscopies	Hyperspectral and FLIM analysis for indirect analysis of embryo metabolism (and potentially the epigenetic state)	Manual/automated	Decreased time to pregnancy by being able to assess the inherent viability of embryos
	Optical coherence tomography to sort and grade cumulus oocyte complexes during collection	Manual/automated	Decreased time to pregnancy by being able to assess the inherent viability of oocytes and hence resultant embryos
Non-invasive biomarkers of health and viability	Analysis of spent culture medium to determine: the metabolic state of embryos and the secretion of specific factors related to viability	Manual/automated	Decreased time to pregnancy and improving our ability to select the healthiest embryo for transfer, thereby looking to ensure the health of the child born
	Analysis of the composition of microvesicles and their cargo	Manual/automated	Increased understanding around implantation, plausibly leading to an increase in implantation rate
Non-invasive genetic diagnosis	Analysis of spent culture medium to quantitate cell-free DNA	Manual/automated	The potential to circumvent the need for blastocyst biopsy, thereby helping to reduce embryo trauma and standardize analysis
Artificial intelligence	Creation of selection aids for spermatozoa	Automated	Increased fertilization rates by the identification of the most viable spermatozoa
	Creation of selection aids for embryos	Automated	Decreased time to pregnancy and reduction in the variability associated with the subjective scoring of embryos
	Digital oversight of quality management	Automated	Improved efficacy of the laboratory

ART, assisted reproductive technology; FLIM, fluorescence lifetime imaging; ICSI, intracytoplasmic sperm injection.

WORDS OF CAUTION AND HOPE

Although this author has no doubts that we shall witness an evaluation, validation and gradual increase in the adoption of several of the technologies listed in TABLE 1, no one technology should be perceived as a panacea. Rather, successful outcomes in IVF always require a holistic perspective of patient treatment and the ART laboratory (*Gardner and Lane, 2003*), reinforcing the adage that you are only as strong as the weakest link. Gone are the days of the ART laboratory being established in the only available space left in the building (such as the aforementioned janitor's closet, basements etc.), but rather there is now an understanding that they need to be centre stage in the creation of new programmes designed for purpose and built to new and accepted standards, i.e. with the health of the gametes and embryos in mind.

Similarly, the laboratory does not operate in isolation: it works in synergy as part of a team with experienced physicians, nurses, counsellors, etc. History has shown that exceptional ART programmes were (and are) those where effective and respectful teams are established.

Finally, increasing the amount of technology (and the automation that will follow) in the ART laboratory will not mean a demise in the career of the embryologist. Far from it. Based on the premise that 'The way to improve ART outcomes is to introduce more technologies in the laboratory', progress will ensure that the growing demands for ART are met successfully. Although it may indeed come to pass in the years to follow that fewer embryologists are required per cycle, as less time will be spent performing skilled technologies such as ICSI and cryopreservation, the increasing demand for ART worldwide and growth in the number of cycles will ensure that embryologists will always be necessary and will remain fundamental in the success of an ART programme.

REFERENCES

Bormann, C.L., Curchoe, C.L., Thirumalaraju, P., Kanakasabapathy, M.K., Gupta, R., Pooniwala, R., Kandula, H., Souter, I., Dimitriadis, I., Shafiee, H. **Deep learning early warning system for embryo culture conditions and embryologist performance in the ART laboratory.** J. Assist. Reprod. Genet. 2021; 38: 1641–1646

Esteves, S.C., Bento, F.C. **Implementation of cleanroom technology in reproductive laboratories: the question is not why but how.** Reprod. Biomed. Online 2016; 32: 9–11

Ferrick, L., Lee, Y.S.L., Gardner, D.K. **Reducing time to pregnancy and facilitating the birth of healthy children through functional analysis of embryo physiology.** Biol. Reprod. 2019; 101: 1124–1139

Gardner, D.K., Lane, M. **Towards a single embryo transfer.** Reprod. Biomed. Online 2003; 6: 470–481

Gardner, D.K. **Mammalian embryo culture in the absence of serum or somatic cell support.** Cell Biol. Int. 1994; 18: 1163–1179

Gardner, D.K., Reineck, P., Gibson, B.C., Thompson, J.G. **Microfluidics and microanalytics to facilitate quantitative assessment of human embryo physiology.** Agarwal A., Varghese A., Nagy Z.P. Practical Manual of In Vitro Fertilization: Advanced Methods and Novel Devices. Humana Press New Jersey 2019: 557–566, 2nd Edition

Lu, Z., Zhang, X., Leung, C., Esfandiari, N., Casper, R.F., Sun, Y. **Robotic ICSI (intracytoplasmic sperm injection).** IEEE Trans. Biomed. Eng. 2011; 58: 2102–2108

Munne, S., Kaplan, B., Frattarelli, J.L., Child, T., Nakhuda, G., Shamma, F.N., Silverberg, K., Kalista, T., Handyside, A.H., Katz-Jaffe, M. **Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial.** Fertil. Steril. 2019; 112: 1071–1079

Nagy, Z.P., Shapiro, D., Chang, C.C. **Vitrification of the human embryo: a more efficient and safer in vitro fertilization treatment.** Fertil. Steril. 2020; 113: 241–247

Tran, A., Cooke, S., Illingworth, P.J., Gardner, D.K. **Deep learning as a predictive tool for fetal heart pregnancy following time-lapse incubation and blastocyst transfer.** Hum. Reprod. 2019; 34: 1011–1018

Received 14 October 2021; accepted 19 October 2021.