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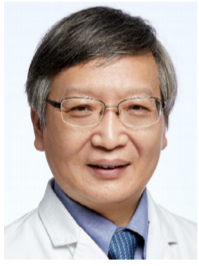
ARTICLE

A compact, high-throughput semi-automated embryo vitrification system based on hydrogel



BIOGRAPHY

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KEY MESSAGE

The ART industry demands an innovative automated vitrification system for better quality control and standardization of labour-intensive procedures. The hydrogel-based system achieves a compact, high-throughput solution with efficacy similar to the conventional Cryotop method, marking a significant advancement in the automation of ART.

ABSTRACT

Research question: What is the efficiency and efficacy of the novel Biorocks semi-automated vitrification system, which is based on a hydrogel?

Design: This comparative experimental laboratory study used mouse model and human day 6 blastocysts. Mouse oocytes and embryos were quality assessed post-vitrification.

Results: The Biorocks system successfully automated the solution exchanges during the vitrification process, achieving a significantly improved throughput of up to 36 embryos/oocytes per hour. Using hydrogel for cryoprotective agent delivery, 12 vessels could be processed simultaneously, fitting comfortably within an assisted reproductive technology (ART) workstation. In tests involving the cryopreservation of oocytes and embryos, the system yielded outcomes equivalent to the manual Cryotop method. For example, the survival rate for mouse oocytes was 98% with the Biorocks vitrification system ($n = 46$) and 95% for the manual Cryotop method ($n = 39$), of which 46% and 41%, respectively, progressed to blastocysts on day 5 after IVF. CC-grade day 6 human blastocysts processed with the Biorocks system ($n = 39$) were associated with a 92% 2 h re-expansion rate, equivalent to the 90% with Cryotop ($n = 30$). The cooling/warming rates achieved by the Biorocks system were 31,900°C/minute and 24,700°C/minute, respectively. Oocyte quality was comparable or better post-vitrification for Biorocks than Cryotop.

Conclusions: The Biorocks semi-automated vitrification system offers enhanced throughput without compromising the survival and developmental potential of oocytes and embryos. This innovative system may help to increase the efficiency and standardization of vitrification in ART clinics. Further investigations are needed to confirm its efficacy in a broader clinical context.

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KEYWORDS
Assisted reproduction
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Vitrification

INTRODUCTION

The future of assisted reproductive technology (ART) lies in automation and consequent standardization (*Abdullah et al., 2022*; *Casciani et al., 2021*; *Kushnir et al., 2022*). Since the inception of ART inception, embryo laboratories have been replete with procedures that depend heavily on embryologists' expertise and dexterity. Ranging from semen preparation, oocyte collection, IVF, oocyte denudation and intracytoplasmic sperm injection (ICSI) to embryo culture, cryopreservation and embryo transfer, each process demands the meticulous attention of a highly trained specialist.

To enhance the results, the protocols are refined, often leading to an increase in the number of procedural steps. For instance, assisted hatching (*Li et al., 2016*), application of ICSI to overcome male factor infertility (*Esteves et al., 2018*), artificial collapsing in blastocyst cryopreservation (*Boyard et al., 2022*; *Darwish and Magdi, 2016*) and multistep equilibration processes in oocyte vitrification (*Lai et al., 2015*) exemplify the addition of steps to achieve superior clinical outcomes. Simultaneously, protocol optimization must take user usability into account, potentially limiting further refinement. Furthermore, as protocols grow increasingly sophisticated, necessitating heightened skill, only the most accomplished embryologists at ART centres can consistently reproduce these procedures. The cultivation of embryologist expertise is a laborious process, and the supply struggles to satisfy the escalating demand for ART services. Streamlining these procedures through automation in a high-throughput fashion offers a promising resolution to these challenges.

Several endeavours to automate ART are underway, including the assessment of embryos through deep learning (*Khosravi et al., 2019*; *Tran et al., 2019*), computer-assisted sperm analysis (*Irvine, 1995*; *Krause, 1995*), semen preparation (*Jafek et al., 2020*), sperm sorting (*Schuster et al., 2003*; *Shirota et al., 2016*), oocyte denudation (*Weng et al., 2018*; *Zeringue et al., 2005*, *2001*), embryo culture systems (*Angione et al., 2015*; *Han et al., 2010*; *Heo et al., 2012*; *Kim et al., 2009*), time-lapse imaging (*Cruz et al., 2012*; *Finn et al., 2010*; *Meseguer et al., 2011*), ICSI automation (*Leung et al., 2012*; *Lu et al., 2011*; *Wang et al., 2020*) and automated vitrification (*Arav et al., 2018*; *Liu et al., 2015*; *Miao et al., 2022b*; *Roy et al., 2014*). A recent automated sperm injection (ICSIA) system (Overture Life, Spain) has resulted in birth of the first babies (*Costa-Borges et al., 2023*).

Among these, certain technologies, such as computer-assisted sperm analysis and time-lapse imaging, have reached a mature stage and have been commercialized, while others, such as sperm sorting, oocyte denudation and ICSI automation, are in the stages of proof-of-concept or preclinical study. This discrepancy arises primarily because automations rooted in optical imaging do not modify much of the traditional ART process. The gametes are non-invasively observed by the automated system, using well-established observational methods. However, for systems that need physical manipulation of the gametes, the commercialization and adoption rates are considerably lower.

One rationale is that these technologies are inherently more sophisticated, making it a challenging task to engineer them into user-friendly, resilient, high-performing, efficient and cost-effective systems that fulfil all clinical needs. Another consideration is that these innovative techniques are currently under investigation only in animal models, in which the gametes differ significantly from human gametes in size, cytoplasmic integrity and resistance to various processes.

In particular, the cryopreservation of human gametes is an indispensable but labour-intensive technique in ART clinics. Vitrification, a rapid cryopreservation method, replaced slow-freezing as the preferred technique for cryopreserving oocytes and embryos. Unlike slow-freezing, which prevents intracellular but permits extracellular crystal formation, vitrification aims to prevent both intra- and extracellular crystallization. To accomplish this, both a high concentration of cryoprotective agent (CPA) and a high cooling rate are necessary to enable liquid to transition into an amorphous solid state.

The solutions with high concentrations of CPA carry a risk of high osmolarity and toxicity. In order to mitigate osmotic and chemical damage, various CPA formulations and operational protocols have been proposed. However, all of them necessitate embryologists to meticulously manipulate the embryo under a microscope, expose it to different concentrations of CPA, and ultimately place it onto a vessel for submersion in liquid nitrogen. The timing of each step is also critical. This is a laborious, manual task and heavily reliant on the operator's skill for the procedure. As such, the ART industry has long called for an automated vitrification system (*Vajta, 2013*).

Several approaches to automated vitrification are reported in the literature. For instance, Liu and colleagues developed a robotic system that relies on machine vision to track and manipulate the embryos, completely mimicking what embryologists would do in an ART centre (*Liu et al., 2015*). Another approach involves the use of a microfluidic chip to hold the embryos in place and control the flow of CPA around them (*Guo et al., 2019*; *Heo et al., 2011*; *Lai et al., 2015*). The advantage of this approach is that the CPA delivery profile can be precisely tuned with microfluidic technology. However, connecting the chip to a syringe pump, loading the embryo onto the chip and retrieving the embryo from the chip require extensive training.

Electrowetting has also been proposed to automate this process (*Pyne et al., 2014*), although there are concerns about exposing oocytes and embryos to such a strong electric field. Gavi (Genea Biomedx, Australia) is the only system that has been commercially launched. It is an automated pipette system that moves equilibrium solution and vitrification solution around the embryo to complete the CPA delivery process (*Roy et al., 2014*). Despite its innovative approach, the system's capacity to process only four vessels in parallel may pose constraints for busy ART clinics. Moreover, there is room for improvement in its compatibility with existing storage systems and the compactness of the instrument itself. In summary, there is a need for a high-throughput, user-friendly and compatible automated vitrification system whose vessels can fit into the current storage system.

Recently, Biorocks Company Limited (China) has developed a semi-automated vitrification system in which the CPA is made in the form of a hydrogel. To the best of the current authors' knowledge, this is the first report of a system that uses the properties of a hydrogel for CPA delivery purposes. With this novel

approach, the embryos/oocytes can be placed in the cryopreservation vessel before exposure to the CPA, eliminating the need to transfer the embryos/oocytes to the vessel after the CPA treatment (*Liu et al., 2015*; *Miao et al., 2022b*; *Pyne et al., 2014*). The vessels share a similar size and geometry to the commonly used straws and Cryotop vessel (Kitazato, Japan), which favour the high cooling and warming rate, and also clinical adoption in terms of the liquid nitrogen storage system currently used in ART centres. The instrument can fit into the IVF workstation, enabling the embryologist to complete the entire vitrification process inside the workstation. The system can process 12 vessels simultaneously and has four independent channels.

To evaluate the efficacy of the Biorocks vitrification system, preliminary tests were conducted using mouse oocytes and embryos, as well as CC-grade human blastocysts classified according to the Gardner and Schoolcraft grading system (*Gardner, 1999*) on day 6. To assess the effects of using the Biorocks system on oocytes and embryos, a series of comparative analyses were conducted on mouse material. For mouse oocytes, the following were conducted: (i) IVF to assess the potential to develop to the blastocyst stage; and (ii) fluorescence staining experiments on meiotic spindles, chromosomes and mitochondria to assess oocyte quality. Mitochondria are one of the most important organelles in the cytoplasm, and their dysfunction or abnormality may injure the oocytes' developmental potential (*Gualtieri et al., 2009*; *Lei et al., 2014*). For mouse embryos, an embryo transfer experiment was conducted to evaluate the effects of the system on the embryos' implantation and in-vivo development. These evaluations served as part of the initial study prior to conducting a randomized clinical trial.

MATERIALS AND METHODS

Collection, culture and grouping of mouse oocytes and embryos

The study involving mouse oocytes and embryos was conducted in compliance with the guidelines of the Experimental Animals Management Committee (Jiangsu Province, China). Female B6D2F1 mice were housed in a room under conditions of controlled lighting and humidity, with unrestricted access to food and water. Each female mouse (aged 6–8 weeks) was administered 5 IU of pregnant mare's serum gonadotrophin (Sigma, USA). Then, 48 h later, each female mouse was injected with 5 IU of human chorionic gonadotrophin (Sigma, USA). To collect the oocytes, the female mice were euthanized the following morning and their oviducts dissected. A total of 130 oocytes were collected, 46 of which were vitrified using the Biorocks system, 39 were vitrified using the manual Cryotop method and 45 were categorized as fresh control oocytes. The oocytes were vitrified 2 h after collection.

To collection the embryos, 6- to 8-week-old ICR female mice were superovulated as above. They were subsequently mated with male ICR mice of proven fertility. The following morning, the female mice were inspected for the presence of copulatory plugs. If plugs were present, the females were euthanized, and the oviducts were dissected to collect the zygotes. These zygotes were cultured in 30 μl drops of potassium simplex optimized medium (KSOM) (Sigma, USA), overlaid with mineral oil (Sigma, USA) for 24–72 h in a CO_2 incubator (37.0°C, 5% CO_2, 5% O_2; ESCO, Singapore). This process enabled a total of 752 embryos to be obtained at different timepoints: 256 at the 2-cell stage, 277 at the 6- to 8-cell stage, and 219 at the blastocyst stage.

The cleavage-stage embryos were divided into three groups. The first group comprised 88 embryos at the 2-cell stage and 103 embryos at the 6- to 8-cell stage, which were vitrified using the Biorocks system. The second group served as an untreated fresh control and included 96 embryos at the 2-cell stage and 96 embryos at the 6- to 8-cell stage. The third group comprised 72 embryos at the 2-cell stage and 78 embryos at the 6- to 8-cell stage, which were vitrified using the manual Cryotop method. The blastocyst-stage embryos were divided into two groups: one vitrified by the Biorocks system (150 embryos) and the other by the Cryotop method (69 embryos). The blastocysts were artificially collapsed using a laser system (Hamilton Thorne, USA).

The oocytes/embryos obtained from different mice were categorized according to their stage and were then randomized before being separated into different groups. The fresh embryos at each stage were simply left in culture and counted at relevant stages, with the vitrified counterparts being left in storage for a week and cultured with counting at relevant stages upon warming. The fresh oocytes were left in culture for about 4 h while the vitrified counterparts went through vitrification, storage (1 h) and the thawing process within 5 h, so that all oocytes could go through IVF using the same sperm sample. Then oocytes of each group were counted at the relevant stages.

Human blastocyst collection

For this preliminary study, human blastocysts were collected on day 6 and were classified as grade C for both inner cell mass and trophectoderm, according to Gardner's grading system (*Gardner, 1999*). These were designated as not to be used for treatment and all the patients agreed to their substandard (CC grade) embryos being used for scientific research. In total, 69 such blastocysts were collected. These blastocysts of comparable quality were divided into two groups. The first group, comprising 30 blastocysts, underwent vitrification using the Cryotop vitrification medium and the Cryotop vessel. The second group, consisting of 39 blastocysts, was vitrified using the Biorocks vitrification system. Independent ethical approval for this study was obtained from the Nanjing Drum Tower Hospital Research Ethics Committee (no. 2022-670-01, approval date 22 November 2022).

Description of the Biorocks vitrification system

The Biorocks semi-automated vitrification system comprises five main components: (i) the instrument; (ii) the substrate; (iii) the vessel holder; (iv) the vessel; and (v) the chip (FIGURE 1). The vessel, akin to a Cryotop in size, has a stick-like form. A specialized well is located at its front end, designed to house the embryo (FIGURE 1e). Each vessel is designed to accommodate one embryo, and each vessel has a sheath on it. The vessel holder plays a crucial role in grouping up to three vessels together, and is positioned on the substrate. The chip, which carries the cryoprotectant, is located above the vessel and rests on the substrate (FIGURE 1f). This entire substrate assembly is subsequently inserted into the instrument to conduct the cryoprotectant delivery process (FIGURE 1g). Each instrument is equipped with four independent channels, with each channel able to accommodate one substrate. Consequently, the system is capable of processing up to 12 vessels simultaneously.

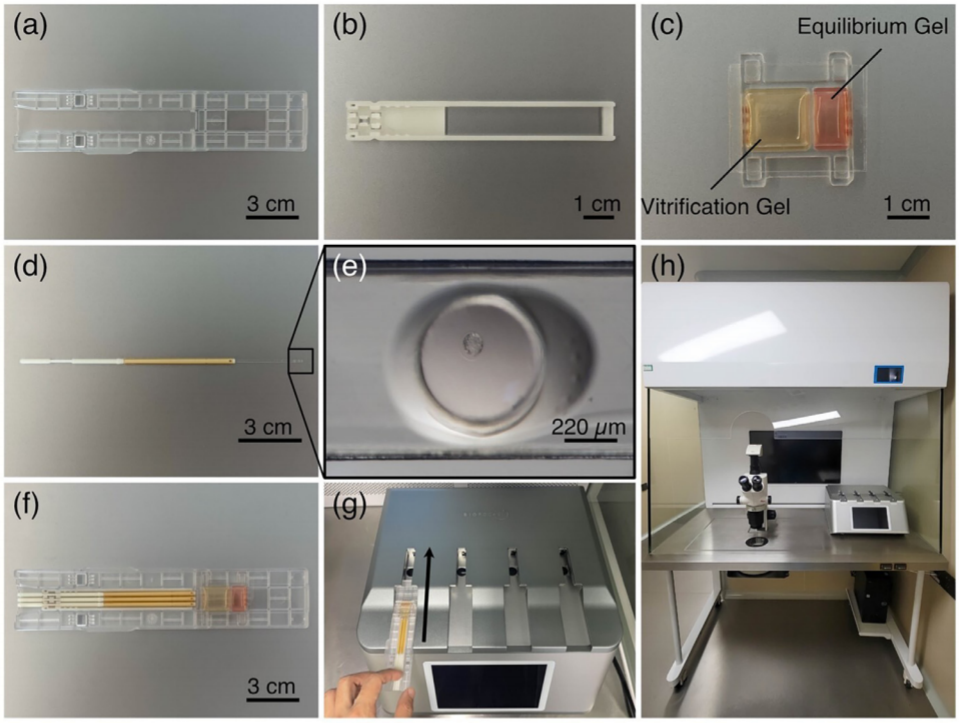

FIGURE 1 The Biorocks semi-automated vitrification system. (a) Image of the substrate, which functions as a platform for loading samples and consumables into a channel of the instrument. (b) Image of the vessel holder, which is designed to accommodate between one and three vessels together during the vitrification process. (c) Image of the chip, which contains the equilibrium and vitrification gel within its chambers. The hydrogel serves as a source for the delivery of the cryoprotective agent. (d) Image of the vessel. Each vessel is designed to accommodate a single oocyte or embryo within a well at its front end. (e) Magnified image of the well of the vessel, showing a mouse blastocyst positioned within the well. (f) The assembled set of consumables including the substrate, vessel holder, three vessels and chip. (g) The image of the instrument depicts a user inserting an assembled set of consumables to process three vessels on one channel (indicated by the arrow). The instrument is equipped with four independent channels, enabling it to process up to 12 vessels simultaneously. (h) The image of a typical set-up of the Biorocks vitrification system within an ART workstation. The instrument measures 41 cm in width, 39 cm in depth and 19.5 cm in height.

The basic solution and the chip (containing equilibrium solution gel and vitrification solution gel) were supplied by Biorocks (China). The hydrogel, which contains equilibrium solution and vitrification solution) is agarose gel. Agarose gel is very stable, chemically and physically, and has wide application in various fields, such as electrophoresis to resolve DNA fragments, enzyme encapsulation and the food industry (*Jiang et al., 2023*)

Detailed description of the Biorocks vitrification process

The research model of the Biorocks vitrification system was used in this study. Initially, the instrument power was switched on and the relevant protocol selected based on the type of samples being processed. Basic solution (500 μl), comprising a HEPES-buffered culture medium without CPA, was added to an organ culture dish. Three vessels were then secured on the vessel holder, which was subsequently situated on the substrate. Subsequently, a small volume of basic solution from the organ culture dish was transferred into the well of each vessel under a stereoscope. Following this, the embryos or oocytes designated for processing were transferred from their respective culture dishes to the basic-solution-containing organ culture dish. Here, a washing step was implemented to rid the embryos or oocytes of any residual culture medium.

Afterwards, the embryos or oocytes were loaded into the wells of the vessels. Upon loading three embryos or oocytes into three respective vessels, a chip encapsulating the CPA in hydrogel form was positioned over the vessel, ensuring it did not make contact with the well. The substrate hosting the chip and vessels was then inserted into the instrument and the predetermined protocol was initiated.

The primary function of the instrument was to exert force onto the chip, causing it to move over the vessels. The chip housed two chambers: one chamber contained the equilibrium gel and the other stored the vitrification gel. Upon initiating the protocol, the equilibrium gel made contact with the well for approximately 14 min, followed by the vitrification gel, which was in contact for around 60 s when processing the blastocysts. The gels, when in contact with the well, facilitated the diffusion of the CPA from the gel into the well, effectively delivering the CPA to the embryos or oocytes. Once the protocol was complete, the vessel holder – now holding the three vessels – was promptly removed from the substrate and instantly plunged into liquid nitrogen, which required a manual operation. After the assembly had been plunged into the liquid nitrogen, the sheaths (already on the vessels) needed to be pulled down with tweezers. The samples were then ready for storage.

Description of the manual vitrification process (manual Cryotop method)

For the manual vitrification process for mouse oocytes and embryos and human

blastocysts, the Cryotop method was used, employing Cryotop vitrification media. Briefly, mouse oocytes were placed in a 20 μl drop of basic solution. Following this, 20 μl of equilibrium solution was introduced to the drop containing the oocytes and this was left for 3 min. Another 20 μl of equilibrium solution was added for an additional 3 min. Subsequently, 240 μl of equilibrium solution was added and the oocytes were left for 9 min. The oocytes were then transferred to 300 μl of vitrification solution for a brief period of 30 s, followed by another transfer to a fresh 300 μl of vitrification solution for another 30 s. In the final step, the oocytes were set on the Cryotop with a minimal droplet volume and immediately plunged into liquid nitrogen. For the mouse embryos and human blastocysts, the initial step involved placing them in 300 μL of equilibrium solution for 10 min. Afterwards, they followed the identical procedure to the oocytes, being transferred to the vitrification solution and then onto the Cryotop, and ultimately being plunged into liquid nitrogen.

Description of the manual warming process

Oocytes or embryos, whether vitrified by the Biorocks system or the Cryotop method, went through the same warming process using Cryotop warming media (Kitazato, Japan). Thawing solution (1 ml) was warmed in an incubator set to 37°C without a CO_2 supply for 30 min. Diluent solution (300 μL) was moved to an organ culture dish and 600 μl of washing solution was put into two organ culture dishes — 300 μl of washing solution in each. When it came to warming the oocytes or embryos, the protective tube was removed from the Biorocks or Cryotop vessel under liquid nitrogen. The vessel was then immersed in 1 mL of 37°C thawing solution. After 1 min, the oocytes or embryos were transferred to the diluent solution for 3 min and to the washing solution for 5 min. Next, the oocytes or embryos were moved to another dish of washing solution for 1 min. The oocytes and embryos were washed in drops of culture medium several times before finally placing them in a CO_2 incubator for further culture and assessment.

Description of the mouse oocyte IVF process

Two male mice (B6D2F1, 12 weeks old) were euthanized by cervical dislocation. The caudae epididymides were collected and the spermatozoa were incubated in human tubal fluid (Sigma, USA) covered with light mineral oil (Sigma, USA) at 37°C for 1 h to induce capacitation. The following experiments and assessments were conducted on three groups of oocytes (see 'Collection, culture and grouping of mouse oocytes and embryos' for a description of these). The fresh control was subjected directly to IVF without undergoing the vitrification process. Conversely, the two vitrified groups were warmed and subsequently cultured for 2 h prior to IVF fertilization. Fertilization was performed by adding 400–800 spermatozoa/μl into the human tubal fluid containing the (warmed) oocytes and cultured in a CO_2 incubator (37.0°C, 5% CO_2, 5% O_2; ESCO, Singapore) for 4 h. Subsequently, the oocytes underwent two washes in pre-equilibrated KSOM medium and were further cultured in an oil-overlaid dish, where groups of 15 oocytes each were placed in 30 μL drops of KSOM, with a culture duration of 96 h. The outcomes were evaluated by measuring the 2-cell rate, the blastocyst formation rate and the hatching rate.

Mouse embryo transfer and implantation evaluation

Embryos at the 4-cell stage obtained from B6D2F1 mice were used for the transfer experiments (Supplementary Materials and Methods). The embryos were categorized into three groups: fresh controls, embryos vitrified using the Biorocks system, and embryos vitrified with the manual Cryotop method. Ten embryos from each group were then transferred to the oviducts of 0.5-day pseudo-pregnant recipient B6D2F1 female mice (6–8 weeks) mated with vasectomized B6D2F1 male mice. Each group used a single pseudo-pregnant female. The count of healthy offspring was evaluated following a gestation period of 19–21 days.

Quality assessment of mouse oocytes

Structural preservation of the oocytes was evaluated by the number and structure of meiotic spindles and chromosomes, as well as the polarization level of the mitochondria. The organelles were observed after fluorescence staining. The following experiments and assessments were conducted on three groups of B6D2F1 mouse oocytes — oocytes vitrified by the Biorocks method, those vitrified by the Cryotop method, and fresh control embryos. For the groups that underwent vitrification, the oocytes were cultured for 2 h in a CO_2 incubator (37.0°C, 5% CO_2, 5% O_2) before undergoing the treatment described below. Oocytes used for quality assessment went through the same grouping and culture procedures as for the survival experiments.

Spindle and chromosome assessment

Oocytes were initially fixed with 4% paraformaldehyde (Yeasen, China) for 30 min at room temperature (22°C). After thorough rinsing, the oocytes were permeabilized in a phosphate-buffered saline (PBS) solution containing 0.1% Triton X-100 and 1% bovine serum albumin (BSA; Yeasen, China) for 20 min. The oocytes were then incubated with an Alexa Fluor 488-conjugated anti-α-tubulin monoclonal antibody (C1051S; Beyotime, China) in a PBS medium, also supplemented with 0.1% Triton X-100 and 1% BSA, and maintained at room temperature for 1 h. Chromosomal staining was achieved using 4′,6-diamidino-2-phenylindole (DAPI). Following additional rinses, the oocytes were placed into a glass-bottom dish containing PBS in preparation for imaging.

Observations were conducted utilizing a fluorescence microscope equipped with a 60× objective lens. The tubulin and chromatin structures were visualized using fluorescein isothiocyanate (FITC) and DAPI filter sets, respectively. Image acquisition encompassed multiple focal planes and was executed in two separate channels. Subsequent analysis focused on the number, morphology and spatial arrangement of the spindles and chromosomes. Spindles with a barrel-shape were regarded as normal, while partial or total disorganization of the microtubules was regarded as abnormal (*Lei et al., 2014*; *Li et al., 2006*). Chromosomes located at the equatorial region of the spindle as a compact metaphase plate were regarded as normal, and other configurations as abnormal (*Lei et al., 2014*; *Li et al., 2006*).

Mitochondrial assessment

Mitochondrial function is usually assessed by the inner membrane potential, $\Delta\Psi_m$, which can be assessed by staining with JC-1 and evaluated by the corresponding ration of red to green fluorescence intensity. With an inner mitochondrial membrane potential $\Delta\Psi_m < 100$ mV, the mitochondria show low polarization; JC-1 remains a monomer and emits green fluorescence in the FITC channel. With $\Delta\Psi_m > 140$ mV, the mitochondria show high polarization; JC-1 forms an aggregate and

emits red fluorescence in the CY3 channel. The ratio of red to green fluorescence intensity represents the polarization of the mitochondria.

Oocytes were cultured in KSOM containing JC-1 (Yeasen, China) for 30 min at 37°C in a CO_2 incubator. Following incubation, they were rinsed and subsequently incubated in HEPES-buffered CZB medium. Observation was performed under a fluorescence microscope, employing a 20× objective lens. For the detection of fluorescence, an FITC filter set was used to capture the green emission, while a CY3 filter set was used for the red emission. To ensure consistency of comparative analysis, images across all three experimental groups were captured using identical camera settings. The quantitative fluorescence intensity of both the green and red signals was determined by computing the total intensity within the region corresponding to each oocyte. This assessment was carried out via image processing algorithms implemented in Python (version 3.11, https://www.python.org/).

Measurement of cooling and warming rates

The cooling and warming rates of the Biorocks vessel were measured using a type T thermocouple with a 0.2 m gauge (Kaipusen, China), and the data were collected using a data acquisition module (Xunyan Electronic, China) at a frequency of 10 Hz. The thermocouple probe was inserted inside the vessel's well, where the embryo would be placed. For the measurement of cooling rate, Cryotop vitrification solution was transferred to the well with a Pasteur pipette to cover the probe. The vessel was then plunged into liquid nitrogen with a slight stirring motion. After the temperature had stabilized, the vessel was removed from the liquid nitrogen to measure the warming rate. This involved inserting the vessel into an organ culture dish containing 1 ml of 37°C Cryotop thawing solution.

Statistical analysis

The chi-squared test was employed for the statistical analyses to compare the vitrification outcomes of mouse oocytes and embryos between the Biorocks system, the manual Cryotop method and the fresh controls, the vitrification outcomes of human blastocysts between the Biorocks system and the Cryotop method, as well as the outcomes of spindle and chromosome evaluation among the three groups. This method was used unless an expected cell count was less than 5. In such cases, a Fisher's exact test was applied. Analysis of variance (Bonferroni's test as a post-hoc test) was employed on the statistical analyses of mouse oocyte mitochondrial outcomes. The level of significance for all the tests was set at $P < 0.05$, and all statistical analyses were carried out using SPSS 21.0 (SPSS Inc., USA).

RESULTS

Mechanism and evaluation of the Biorocks vitrification system for mouse oocytes and embryos

The unique and most inventive difference between the Biorocks system and other (semi-)automated systems described in the scientific literature is that the Biorocks system employs the cryoprotectant solution in the form of a hydrogel. The basic solution within the vessel's well serves as a liquid bridge between the embryo and the cryoprotective gel. When the chip is moved by the instrument, the hydrogel inside contacts the vessel's well, and the CPA inside the gel diffuses into the basic solution and ultimately reaches the oocytes or embryos.

The chip has two chambers containing the equilibrium gel and vitrification gel, respectively. These gels serve the same function as the equilibrium and vitrification solutions in the conventional Cryotop method. The embryo equilibrates with the equilibrium gel for 14 min in the case of blastocysts and dehydrates with the vitrification gel for 1 min. Thus, the Biorocks system adheres to the fundamental principles of the Cryotop method. Upon completion of the protocol, the embryo remains within the vessel, surrounded by less than 0.1 μl of solution, ready for immediate immersion into liquid nitrogen. This design removes the potentially complex step of transferring the embryo to a different vessel after exposure to the vitrification solution, which can be challenging for inexperienced technicians who need to ensure a minimal volume of vitrification solution. As a result, the time of exposure to the vitrification solution is minimized, optimizing the procedure's efficiency.

The performance of the Biorocks system was tested using mouse oocytes, cleavage-stage embryos (2 cells and 6–8 cells) and blastocysts. Mouse embryos were collected and cultured to the desired stage, vitrified and warmed as per their grouping, and assessed. The results are shown in TABLE 1.

For the oocytes, 46 oocytes were vitrified with the Biorocks system and 39 using the manual Cryotop method. According to morphological assessment, 45 of 46 (98%) oocytes survived in the Biorocks group and 37 of 39 (95%) in the Cryotop group. After the vitrified oocytes had been warmed and cultured for 2 h, the two vitrified groups together with a fresh group of 45 oocytes went through the same IVF process. For the Biorocks group, 26 oocytes out of 46 (57%) successfully developed to the 2-cell stage, 21 (46%) developed into blastocysts, and 12 (26%) were hatching or hatched by day 5. For the fresh controls, 29 oocytes out of 45 (64%) successfully developed to the 2-cell stage, 25 (56%) developed into blastocysts, and 15 (33%) were hatching or hatched by day 5. For the Cryotop group, 20 oocytes out of 39 (51%) successfully developed to the 2 cell stage, 16 (41%) developed into blastocysts, and 9 (23%) were hatching or hatched by day 5. No statistically significant differences were found.

For the 2-cell embryos, all 88 survived (100%) after the Biorocks vitrification process. A total of 79 (90%) developed to the expanded blastocyst stage, and 65 (74%) were hatching or hatched by day 5. For the fresh control group, 84 out of 96 (88%) 2-cell embryos developed into expanded blastocysts, and 76 out of 96 (79%) were hatching or hatched by day 5. For the 2-cell embryos going through the manual vitrification process using the Cryotop, 71 out of 72 (99%) survived after vitrification, 65 (90%) developed into blastocysts, and 46 (64%) were hatching or hatched by day 5. No statistically significant differences were found.

For 6- to 8-cell embryos, 101 out of 103 (98%) showed 100% of blastomeres intact after the Biorocks vitrification process. The two embryos showing degenerated blastomeres had more than 50% of intact blastomeres. Among these embryos, 96 (93%) developed to the expanded blastocyst stage, and 76 (74%) were hatching or hatched by day 5. For the fresh controls, 89 out of 96 (93%) 6- to 8-cell embryos developed into expanded blastocysts, and 68 out of 96 (71%) were hatching or hatched by day 5. For 6- to 8-

TABLE 1 EVALUATION OF THE BIOROCKS SYSTEM FOR MOUSE OOCYTES AND EMBRYOS

Embryonic measurement	Fresh	Biorocks	Cryotop	*P*-value
Mouse oocytes				
Number	45	46	39	
Survival (100% intact)	–	45 (98%)	37 (95%)	0.296
2 cells	29 (64%)	26 (57%)	20 (51%)	0.467
Blastocyst (day 5)	25 (56%)	21 (46%)	16 (41%)	0.389
Hatching (day 5)	15 (33%)	12 (26%)	9 (23%)	0.552
2-cell embryos				
Number	96	88	72	
Survival (100% intact)	–	88 (100%)	71 (99%)	0.281
Blastocyst (day 5)	84 (88%)	79 (90%)	65 (90%)	0.823
Hatching (day 5)	76 (79%)	65 (74%)	46 (64%)	0.085
6- to 8-cell embryos				
Number	96	103	78	
Survival (100% intact)	–	101 (98%)	77 (99%)	0.502
Survival (≥50% intact)	–	103 (100%)	78 (100%)	–
Blastocyst (day 5)	89 (93%)	96 (93%)	71 (91%)	0.853
Hatching (day 5)	68 (71%)	76 (74%)	49 (63%)	0.270
Blastocysts				
Number		150	69	
2 h re-expansion		143 (95%)	65 (94%)	0.745
24 h re-expansion		149 (99%)	68 (99%)	0.532

Values are *n* or *n* (%).

Outcomes for B6D2F1 and ICR mouse embryos vitrified after automated processing with the Biorocks system compared with the Cryotop method and fresh controls.

The 2-cell rate, blastocyst rate (day 5) and hatching rate (day 5) for oocytes were calculated after IVF.

A chi-squared test was used for comparison, but if the count was less than 5, a Fisher's exact test was used.

–, measurement not applicable.

cell embryos going through the manual vitrification process using the Cryotop, 77 out of 78 (99%) showed 100% of blastomeres intact and all 78 showed >50% of intact blastomeres. Among these embryos, 71 (91%) developed to the expanded blastocyst stage, and 49 (63%) were hatching or hatched by day 5. No statistically significant differences were found.

A total of 150 blastocysts were processed with the Biorocks system and 69 blastocysts using the manual Cryotop method. After warming, blastocyst re-expansion was assessed after 2 and 24 h of culture. For the Biorocks group, 143 (95%) re-expanded within 2 h, and 149 (99%) re-expanded within 24 h. For the Cryotop group, 65 (94%) re-expanded within 2 h, and 68 (99%) re-expanded within 24 h. No statistically significant differences were found.

Throughput

Throughput is a crucial feature of any (semi-)automated vitrification system. The maximum throughput of the Biorocks semi-automated vitrification system was evaluated using mouse blastocysts. All the vessels were pre-labelled, installed in the vessel holder and positioned on the substrate. Furthermore, all the required basic solution dishes were prepared beforehand. During this evaluation, three vessels were processed simultaneously in one channel, each vessel containing a single embryo. The necessary steps for washing and transfer to the vessel's well, described in a previous section, could be completed within 2 min. It is noteworthy that all the system channels operate independently, meaning that there is no mechanical interference between them and they can be individually controlled. As a result, there was substantial flexibility regarding the pacing of the embryo vitrification process.

FIGURE 2 summarizes the proposed workflow for blastocyst vitrification for maximum throughput from the system. The choice was made to process the embryos channel by channel to minimize the time they spent outside the incubator and to provide ample time to plunge the vessel into the liquid nitrogen. Initially, it took 2 min to place the embryos in the vessels' wells and begin the vitrification treatment process in channel 1 (lasting 15 min). Subsequently, a 2 min 'buffering time' was allowed before loading the next group of embryos into channel 2. This buffering time is essential for controlling the pace of vitrification, and the process was repeated until all four channels were engaged. When the protocol in channel 1 had ended, the vessels were plunged into liquid nitrogen and a 1 min buffering time was allowed before loading the next embryo group. This reduction in buffering time from 2 min to 1 min helps to prevent potential conflicts between the embryo loading and vessel plunging times. This pattern was repeated for the remaining channels, creating a full cycle of continuous vitrification.

In this set-up, a single embryologist at one IVF workstation was able to vitrify three full cycles of four channels, totalling 36 vessels, within an hour. It is important to note that this figure is the system's maximum throughput, and the actual throughput will depend on the procedures and pre-vitrification preparations at different ART centres. In addition, less experienced technicians need more time to load the embryos onto the vessels.

Cooling and warming rate

The measured cooling and warming curves of the Biorocks vessel (from −196°C to 22°C) are presented in FIGURE 3. Accordingly, the Biorocks vessel achieved a cooling rate (transitioning from 22°C to −190°C) of 31,900°C/minute and a warming rate (from −190°C to 0°C) of 24,700°C/minute (TABLE 2). As the Biorocks vessel is an open system, it permits direct contact with the liquid nitrogen, thus facilitating high cooling and warming rates.

Mouse embryo transfer

A mouse embryo transfer experiment was conducted to study embryotoxicity or possible long-term detrimental effect of the Biorocks system on embryos' implantation and in-vivo development. Ten embryos at

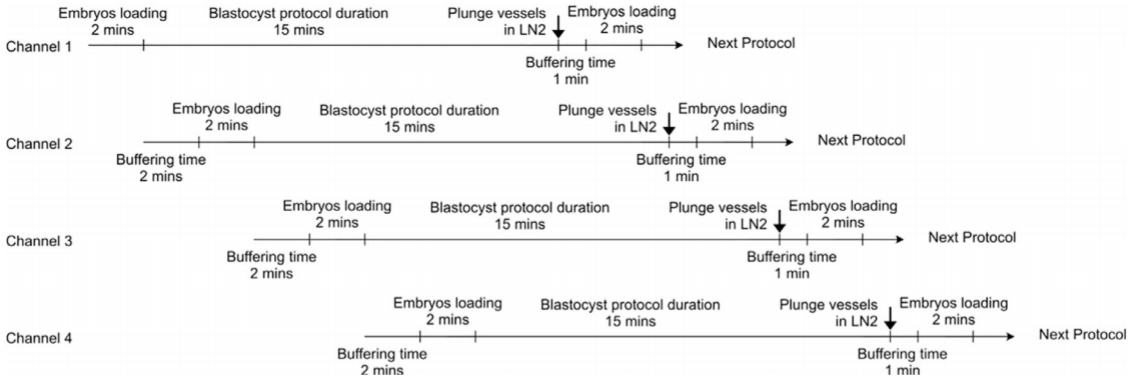

FIGURE 2 Schematic illustration of the workflow of the Biorocks vitrification system, demonstrating its maximum throughput capability of processing 36 vessels per hour, with three independent vessels per channel for each procedure. This workflow is based on the blastocyst vitrification protocol, which takes approximately 15 min to complete. It is important to maintain a steady pace during continuous vitrification processes to prevent any conflicting actions or procedural overlap. LN2, Liquid nitrogen.

the 4-cell were transferred per group (fresh, or previously vitrified using the Biorocks or Cryotop), and the count of healthy offspring was evaluated following a gestation period of 19–21 days. The results showed five healthy offspring each for the fresh control group and the Biorocks group (50% birth rate), while the Cryotop group yielded three (30% birth rate). No observable detrimental effects were observed on any offspring, which were similar in appearance (Supplementary Figure 1).

Assessment of mouse oocytes quality

To further evaluate the effects of using the Biorocks vitrification system on oocytes, the oocytes' structural preservation was also evaluated by fluorescence staining, including the presence, morphology and spatial arrangement of the meiotic spindles and chromosomes, as well as the polarization level of the mitochondria. The results for oocytes vitrified by the Biorocks method were compared with those for the fresh controls and embryos using the manual Cryotop method.

Spindle and chromosome assessment

The spindles and chromosomes were observed by fluorescence microscopy, and classified as normal, deformed or absent; examples of these are presented in FIGURE 4a. As shown in FIGURE 4b, no significant difference existed between the different groups for normal, abnormal or absent spindles or chromosomes.

The detailed results are as follows. For spindles in the fresh control group, 35 out of 45 (78%) oocytes exhibited a normal spindle, 3 (7%) a deformed spindle, and 7 (16%) no spindle. For the Cryotop group, 29 out of 41 (71%) oocytes exhibited a normal spindle, 8 (20%) a deformed spindle, and 4 (10%) no spindle. For the Biorocks group, 22 out of 30 (73%) oocytes exhibited a normal spindle, 5 (17%) a deformed spindle, and 3 (10%) no spindle. In relation to the chromosomes, 40 out of 45 (89%) oocytes in the fresh control group exhibited normal chromosomes, 2 (4%) deformed chromosomes, and 3 (7%) no chromosomes. For the Cryotop group, 37 out of 41 (90%) oocytes exhibited normal chromosomes and 4 (10%) deformed chromosomes. For the Biorocks group, 27 out of 30 (90%) oocytes exhibited normal chromosomes, 2 (7%) deformed chromosomes, and 1 (3%) no chromosomes. No statistically significant differences were found.

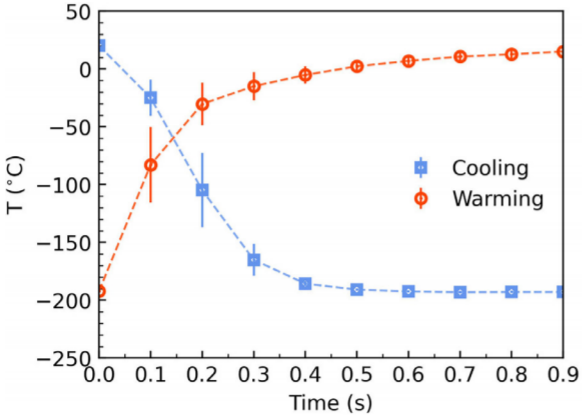

FIGURE 3 Cooling and warming curves of the drops in Biorocks vessel. Each curve represents mean of six trials. The error bar represents the standard deviation. T, temperature.

TABLE 2 COOLING AND WARMING RATE OF THE BIOROCKS VESSEL

Measurement	Cooling	Warming
Mean ± SD	31900 ± 600	24700 ± 2500
Min – Max	31300 – 32800	22500 – 28000

Cooling and warming rates are measured in degrees Celsius per minute (°C/min). Values are based on six repeated measures.

Max, maximum value; Min, minimum value.

Mitochondrial assessment

The ratio of red to green fluorescence was 3.17 ± 0.20 (standard error of the mean) for the fresh control group, 2.62 ± 0.13 for the Cryotop group, and 3.23 ± 0.16 for the Biorocks group. The ratio could be interpreted as the inner membrane potential and indicated polarization. As shown in FIGURE 5b, the ratio of oocytes from the Cryotop group was significantly

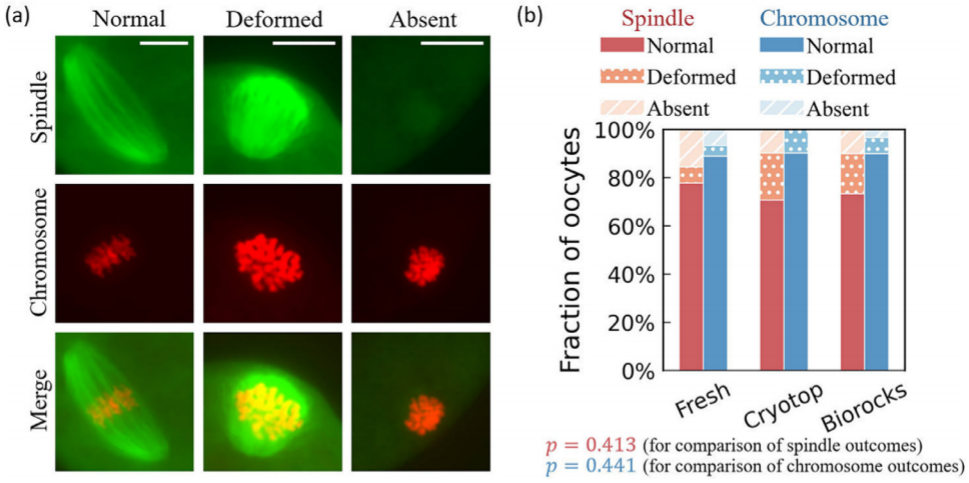

FIGURE 4 Effects of the Biorocks vitrification system on the oocyte spindle and chromosome, observed by fluorescence microscopy. (a) Representative images of normal, deformed and absent spindles (green, α-tubulin stain) and chromosomes (red, DAPI stain) in oocytes. The scale bar is 10 μm. The images in each row were processed in the same way. (b) The fraction of oocytes with normal, deformed and absent spindles/chromosomes in the fresh (n = 45), Cryotop (n = 41) and Biorocks (n = 30) groups. Comparisons of spindle and chromosome data among the three groups employed the chi-squared test; P-values are given in red for spindles and blue for chromosomes.

lower than the Biorocks group (P = 0.017), and no significant differences were found between the fresh control group and the Biorocks group.

Evaluation of the system using human blastocysts

The system's performance was evaluated using suboptimal quality human blastocysts, in addition to mouse oocytes and embryos. For this preliminary study, only blastocysts of CC grade, as per the Gardner grading system on day 6, were collected. A total of 69 blastocysts were vitrified, 39 undergoing vitrification with the Biorocks system and 30 with the Cryotop method. The outcomes revealed that 36 (92%) and 27 (90%) blastocysts re-expanded within 2 h post-warming for the Biorocks system and the Cryotop method, respectively. FIGURE 6 shows the representative images of the human blastocysts before and 2 h after warming. No significant difference was discerned between the two vitrification methods (P = 0.530).

DISCUSSION

Automation and standardization are key trends in the advancement of ART. Over the past two decades, numerous efforts have paved the way to exploring the

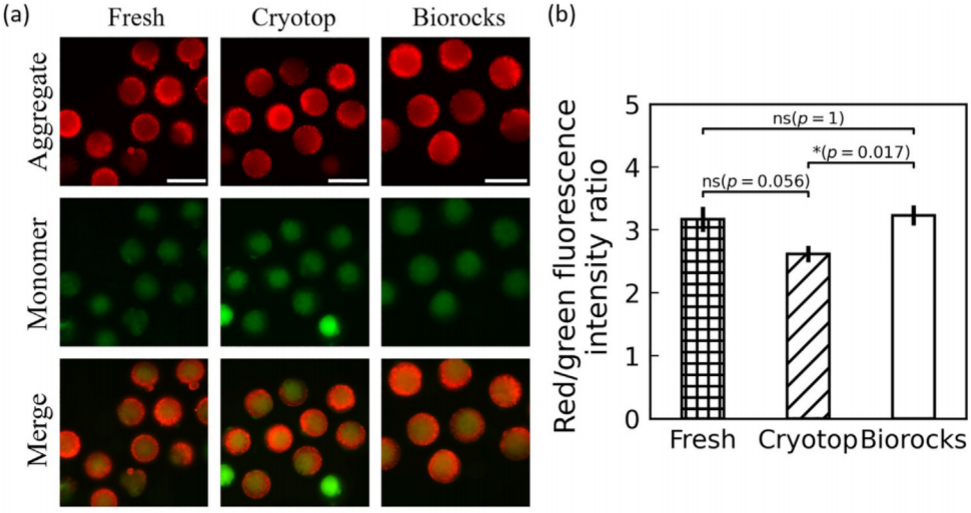

FIGURE 5 Effects of the Biorocks' vitrification system on the inner mitochondrial membrane potential measured by JC-1 fluorescence. (a) Relative inner membrane potential ( ) in oocytes of the Fresh, Cryotop, and Biorocks groups. The scale bar is 100 μm. All pictures were processed in the same way. The degenerated oocytes (ultra bright ones in FITC channel) were not included in subsequent intensity ratio calculations. (b) Red/green fluorescence intensity of the three groups. Data are presented by of three independent experiments. * means statistically significant difference exists ($p < 0.05$).

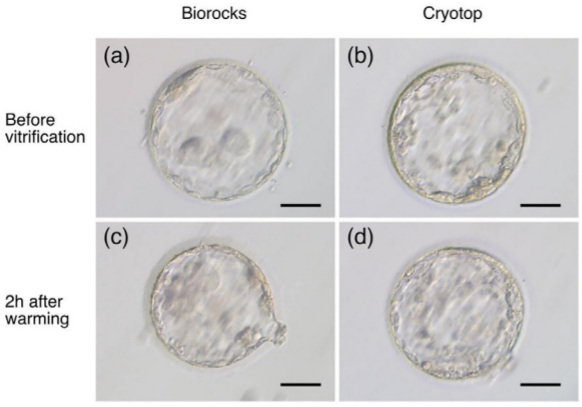

FIGURE 6 **Representative images of human CC-grade blastocysts before vitrification (a, b) and 2 h after warming (c, d). (c) Vitrified by the Biorocks system. (d) Vitrified by the Cryotop method. The scale bar is 50 μm.**

potential benefits of automation in ART. These endeavours have been crucial in demonstrating the feasibility of (semi-) automated technologies, providing a path towards their practical application. However, to make a tangible impact on patients in ART centres, these (semi-) automated systems must reach commercialization, a process that involves a careful balance of efficacy, safety, throughput, usability, cost-effectiveness and mass producibility.

In the realm of (semi-)automated vitrification, the focus lies on the delivery of CPA to the embryo. The mode of delivery largely dictates the characteristics and commercialization potential of the system. Take the classical microfluidics method, for instance. This method excels in controlling the CPA concentration within a microenvironment, enabling vital research into how oocytes respond to varying osmotic stresses (*Heo et al., 2011*; *Lai et al., 2015*, *2013*; *Song et al., 2009*; *Zhao et al., 2017*). However, usability remains a major challenge for the classical microfluidics systems reported in the literature. Users are required to possess specific skills, such as chip conditioning, bubble prevention and fine-tuning of pressure, to ensure optimal results. These skills are rarely found among clinic staff, which has significantly hindered the adoption of this method in ART clinics. Consequently, while the classical microfluidics method offers significant potential, its practical application is hampered by the steep learning curve and specialized skills required for operation.

Another approach involves the use of a robotic arm to physically manipulate the embryo, relying on machine vision for precise tracking (*Liu et al., 2015*). In this set-up, an algorithm directs a Pasteur pipette to carry the embryo through various solutions during the vitrification process. While technologically advanced, this method presents significant challenges for wide-scale adoption. First, it necessitates the use of a costly inverted microscope equipped with a motorized mechanical stage, which may be prohibitively expensive for many clinics. Second, the method essentially mimics human operation, inherently limiting its throughput. The combination of high costs and low productivity compromises the cost-effectiveness of this system, thus hindering its potential for commercialization.

Another method that deserves mention is the one that utilizes a robotic pipette system to manipulate the cryoprotective solution, rather than directly handling the embryo (*Miao et al., 2022b*; *Roy et al., 2014*). A prominent example of this technique is the commercially available Gavi system. Gavi has demonstrated technical viability and provides a user-friendly experience, significantly minimizing the need for intensive training. Despite these advantages, there are a few considerations that may limit its broader adoption in ART clinics. First, the investment needed for the Gavi system might be a concern for some clinics. Moreover, given its size, the system might require thoughtful planning for integration into existing laboratory spaces. Also, the need for its specific vessel (pod) could pose compatibility challenges with the storage systems currently in widespread use. Finally, the system's throughput — the number of samples it can process concurrently — might not always align with the high-volume requirements of busy ART clinics. Therefore, although the semi-automated Gavi system represents a significant and remarkable step towards automated vitrification, it also underscores the ongoing need for an automated solution that is cost-effective, compact, compatible and capable of high-throughput operation.

The Biorocks semi-automated vitrification system offers a novel approach, using hydrogel as a medium for CPA delivery. This significant deviation from the traditional liquid-based delivery systems enables new possibilities in the design of instruments and consumables.

The adoption of a hydrogel eliminates the need for the costly and complex pipette and multi-axis mechanical systems required for solution relocation. Instead, the Biorocks system utilizes a single linear module to move the chip containing the CPA-laden hydrogel, thereby completing the CPA delivery process. This enables the system to include four independent channels in a compact instrument (41 cm in width, 39 cm in depth and 19.5 cm in height) that fits comfortably within an ART workstation (FIGURE 1h). This level of integration facilitates a smooth transition into existing workflows and optimizes the use of the same microscope for embryo transfer and manipulation, and the same IT system for identification and double-checking.

Although the Biorocks system efficiently automates the dehydration process, it remains semi-automatic due to the manual requirements for embryo or oocyte loading and vessel transfer to the liquid nitrogen. Despite this, the immersion of the vessels into the liquid nitrogen is a straightforward task, reducing the urgency for complete automation. Given the space constraints in IVF workstations, the current design of the Biorocks system is practically sized. Full automation would inevitably increase the size of the system. In the future, it is hoped that there will be automated warming or rehydrating procedures, potentially standardizing the entire cryopreservation process even further.

When compared with the Cryotop method, where a proficient embryologist can process approximately eight vessels per hour, the compact Biorocks system

offers a substantial enhancement in throughput. This automation could result in a significant conservation of the manpower and effort previously expended in manual vitrification. ART centres should determine their own pace of operation when using this system. This saving can be further used for critical tasks like data analysis from other systems such as time-lapse incubators, or for executing intricate procedures such as ICSI. Thus, a high-throughput system not only streamlines the vitrification process, but also contributes to overall laboratory efficiency and effectiveness.

Concurrently, the cooling, storage and warming processes are vital components of successful vitrification, along with the CPA delivery process. The vessel employed in these functions is integral to the success of these steps. For vitrification to be successful, the portion of the vessel housing the embryos must have a minimal mass to reduces its heat capacity. This allows for the attainment of a sufficiently high cooling and warming rate. This necessity presents a significant challenge for integrating both the CPA delivery and vessel functions into a single device. As a result, many (semi-)automated systems discussed in the literature necessitate a transfer step – either automated or manual – to move the embryo from the CPA delivery device to the vessel after CPA treatment (*Liu et al., 2015*; *Miao et al., 2022b*, *2022a*; *Pyne et al., 2014*). This transfer process is time-consuming and inadvertently extends the embryo's exposure to the vitrification solution, which is not favourable. It also further complicates the mechanical design of the system, leading to a larger, more costly instrument with limited throughput.

In contrast, the system presented in this report loads the embryo onto the vessel before the CPA delivery process. The embryo remains stationary within the well of the vessel throughout the CPA treatment, effectively eliminating the need for and the risks associated with relocating the embryos. Moreover, the vessel in this system is similar in size and geometry to commonly used straws and Cryotop vessels. This compatibility aligns well with the existing liquid nitrogen storage systems in ART clinics, favouring potential clinical adoption.

Successful vitrification is theoretically favoured by higher cooling and warming rates (*Arav, 2014*). Various vessels available on the market demonstrate a wide range of these rates. For instance, Cryotop, an open system, reports exceedingly high cooling and warming rates of 24,000°C/minute and 42,000°C/minute, respectively. On the other hand, Gavi, a closed system, reports a cooling rate (from 0°C to –133°C) and warming rate (from –133°C to 0°C) of 14,136°C/minute and 11,239°C/minute, respectively (*Roy et al., 2014*). Another closed system, Rapid-i (Vitrolife, Sweden), indicates a cooling rate of 1220°C/minute and a warming rate of 7700°C/minute (*Desai et al., 2013*).

Each of these vessels is commercially available and their efficacy in clinical settings has been demonstrated by various studies. Therefore, while higher values are generally considered favourable, cooling and warming rates ranging from a few thousand to a few tens of thousand degrees per minute are deemed acceptable for clinical applications. The Biorocks vessel falls within this acceptable range, having cooling and warming rates of a few tens of thousand degrees per minute. Potential concerns have been raised about the possibility of infection and disease transfer due to the direct contact with the liquid nitrogen (*Bielanski et al., 2003*, *2000*). However, no cases of disease transfer have been reported to date using the open system in vitrification (*Desai et al., 2013*; *Vajta et al., 2015*). Moreover, one study reported no observation of bacteria or fungi in any of the warming media, irrespective of whether an open or a closed device was used (*Pemán et al., 2016*). For a more detailed discussion of the open and closed systems used in vitrification, readers are referred to a comprehensive review that discusses the benefits and risks of open versus closed systems for vitrification (*Vajta et al., 2015*).

Efficacy is another primary consideration for a successful automated vitrification technology. The current experimental results using mouse oocytes and embryos demonstrate promising outcomes with the Biorocks system. Both oocytes and embryos demonstrated high survival rates post-vitrification and warming. Furthermore, the blastocyst rate and hatching or hatched rate in the experimental group using 2-cell and 6- to 8-cell embryos did not differ significantly from the fresh control group. To figure out the potential effects on oocytes of using the Biorocks vitrification system, IVF experiments were conducted. The calculated 2-cell rate, blastocyst rate and hatching rate showed no statistically significant differences among the Biorocks group, the manual Cryotop group and the fresh control group.

To examine the system's embryotoxicity and its possible effects on embryos' implantation and in-vivo development, a transfer and implantation experiment was carried out on mouse 4-cell stage embryos. No observable detrimental effects were seen in the offspring, indicating that there is no embryotoxicity, although a repeat of the experiments is necessary to confirm this preliminary finding. Furthermore, morphological assessments (of spindles and chromosomes) and functional validations (of mitochondria) on the structural preservation of the vitrified oocytes were conducted using fluorescence staining. The results further validated that the Biorocks system had no extra negative impact on the quality of oocytes compared with the manual vitrification method. In brief, the Biorocks system is not only effective in maintaining the survival of embryos and oocytes during vitrification and warming, but also does not negatively affect their intracellular structure or development potential compared with the manual Cryotop method.

In the case of vitrified blastocysts, the re-expansion rates were notably high at both 2 h and 24 h post-warming, suggesting that the blastocysts vitrified by the Biorocks system retained their potential for growth and development after undergoing the vitrification and warming processes. However, it is important to note that the hatching or hatched rate on day 5 was not evaluated in these blastocysts due to the artificial collapse procedure, which can affect the zona pellucida and consequently influence the hatching rate. Despite this limitation, the overall results from the mouse models provide strong evidence for the efficacy of the Biorocks vitrification system.

Given the promising results demonstrated in the mouse model, the authors initiated a human blastocyst study as a preliminary step towards evaluating the feasibility of deploying the Biorocks system in a clinical environment. To gain regulatory approval, it is crucial to demonstrate the safety and efficacy of this system through well-designed clinical trials. As part of this preliminary investigation suboptimal human blastocysts, graded CC based on the Gardner grading system on day 6,

were specifically selected for vitrification. The current results indicate that the Biorocks system is effective in vitrifying these lower grade blastocysts. However, it is widely acknowledged that high-grade blastocysts demonstrate superior resilience against the stresses associated with vitrification, coupled with improved recovery and re-expansion capabilities following warming. Consequently, the results of this preliminary study may not fully represent the performance of the system in a typical clinical setting, where blastocysts of better quality will be processed.

In view of this, after more preclinical and safety studies have been done, the authors plan to conduct a structured, randomized clinical trial with a substantial sample size to validate the clinical effectiveness of the Biorocks system in the near future. This will provide a more comprehensive evaluation of its performance, potential advantages and suitability for broader adoption in the field of ART.

CONCLUSION

The advancement of ART relies heavily on the development of automation and the related standardization. We are currently in the early stages of industrialization of ART, with traditional artisanal techniques still playing a major role. Even though automated processes may not currently outperform manual ones – a phenomenon similar to the early days of industrialization – it is important to acknowledge that industrialization brings about a wide distribution of products and a reliable foundation for continuous improvement. In the future, automated systems could be capable of handling complex protocols that are impossible to undertake with manual processes. The process of vitrification automation demonstrates another potential application for automation in ART.

The introduction of the Gavi system, soon to be followed by the Biorocks system, signifies an exciting phase. Notably, the Biorocks system introduces a novel approach to use hydrogel for CPA delivery purposes, which allows for a compact, high-capacity system. The system has already shown impressive results in the mouse model and suboptimal human blastocyst vitrification. The oncoming industrialization of ART is an exciting prospect that promises to improve ART practices and extend its benefits to more patients worldwide, thanks to these technological advancements.

DATA AVAILABILITY

Data will be made available on request.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.rbmo.2023.103769.

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