Scientific Research Publishing

Advances in Reproductive Sciences, 2025, 13(3), 153-161
https://www.scirp.org/journal/arsci
ISSN Online: 2330-0752
ISSN Print: 2330-0744

First Use of a Modified Specific Gravity Device to Determine the Viability and Development Potential of Human Cryopreserved Embryos

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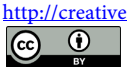
How to cite this paper: Prien, S., Brooks, A., Ahmad, K., Suh, B., and Penrose, L. (2025) First Use of a Modified Specific Gravity Device to Determine the Viability and Development Potential of Human Cryopreserved Embryos. *Advances in Reproductive Sciences*, **13**, 153-161.
https://doi.org/10.4236/arsci.2025.133013

Received: July 10, 2025
Accepted: August 12, 2025
Published: August 15, 2025



Abstract

Objective: Embryo cryopreservation is currently the only effective means for long-term storage, transport, and genetic testing of human and animal embryos. It also allows significant flexibility in scheduling ART procedures in fertility clinics. However, cryopreservation can also lead to embryo damage, affecting embryo viability and health. Previous research from this lab has demonstrated that a Modified Specific Gravity Device (MSGD) can be used to determine viability status and to predict the future development of embryos in various animal species. The current study describes the first use of the MSGD to determine post-thaw viability and development potential of donated cryopreserved human blastocyst stage embryos. **Materials and Methods:** A series of 192 vitrified blastocyst stage embryos (both non-biopsied and biopsied) from 44 patients were donated for research. After some initial testing, it was determined that only non-biopsied embryos should be passed through the MSGD to determine their buoyancy and drop time. Therefore, only 21 straws containing 27 non-biopsied embryos were used in this study. Embryos were first thawed using standard clinical laboratory techniques. Once thawed, the diameter of the zona pellucida of each embryo was estimated by using a microscope lens micrometer, the re-expansion status of the embryos was noted, the embryo was then dropped through the MSGD and placed in culture for 3 hrs at 37˚C. After 3 hrs, each step was repeated and then embryos were returned to culture for 24 hrs to determine hatching. Statistical analyses were made comparing embryos that expanded by 3 hrs and hatched by 24 hrs to their descent time through the MSGD. **Results:** To date, we have completed data collection for 27 embryos. It was observed that most embryos at the initial time point did

not demonstrate any expansion, and no differences were seen in their initial drop times (P = 0.754). However, at 3 hrs in culture, re-expanded embryos dropped more slowly through MSGD than those that did not expand (P < 0.04). Embryos that hatched had faster drop rates at both the initial and 3 hr measurement (P < 0.001 and P < 0.05), most likely due to concentration differences between cellular constituents and the media environment. **Conclusion:** Differences seen continue to suggest that a simple test of buoyancy may allow the determination of embryo viability and development potential. Further study is needed with a much larger embryo population to confirm these observations.

Keywords

Embryos, Buoyancy, Noninvasive Testing, Blastocyst, Selection

1. Introduction

Assisted reproductive techniques (ART) have become the gold standard of infertility treatment, with approximately 2% of the United States birth rate being attributed to the use of ART over the last several years [1]. The last few decades have seen significant improvements in ART stimulation [2], culture techniques [3] [4], and timing of embryo transfers [5]. It has also seen the rise of pre-implantation genetic testing (PGT), which allowed the identification and prevention of the transfer of aneuploid embryos [6], all in the quest to improve take-home baby rates.

However, until very recently, besides PGT, little has changed in terms of assessing the quality of individual embryos selected for the transfer procedure. For over four decades, embryologists have relied mainly on the morphological appearance to select embryos for transfer [7]. Recently, this information has begun to be supplemented with morphometrics, but significant disagreement remains over the value of this information in embryo selection [8]-[13].

Previous work from this laboratory has described an alternative means of embryo selection based on a simple modified specific gravity device [14]-[18]. The device and technique have been used to assess both fresh and frozen/thawed embryos across a number of animal species and has been shown effective in selecting which embryos will survive from the earliest stage of development [16], through the freeze/thaw process [17] [18] and continue to develop post-thaw and establish pregnancy [16].

The current study describes the first experience of the system with human cryopreserved embryos, specifically the effectiveness of the MSGD in predicting post-thaw survival and further development under in vitro fertilization (IVF) culture conditions.

2. Materials and Methods

This study was conducted using cryopreserved embryos donated for research

anonymously by patients through informed consent who had completed their fertility treatment at a university-based fertility clinic. Their use in this study was acknowledged by the Institutional Review Board and Institutional STEM Cell Committees and was reviewed and approved by the Vice President of Research's Office, at Texas Tech University Health Sciences Center prior to the start of the study. Potential embryos to be used in the study were cryopreserved using a commercially available media (Blastocyst Fast Freeze kit, Life Global; Trumbull, CT) following the manufacturer's protocol. In brief**,** blastocyst stage embryos created by IVF were cryopreserved by the vitrification method [19]. Blastocysts were sequentially exposed to 3 cryoprotectant solutions containing increasing concentrations of glycerol and ethylene glycol, loaded into cryo straws, and plunged directly into liquid nitrogen for vitrification. All cryopreserved embryos were stored in liquid nitrogen at −196˚C.

For post-thaw embryo culture, embryo GPS dishes (Life Global) were prepared containing Global medium supplemented with 20% serum substitute supplement (SSS, 35 µl/well under mineral oil), equilibrated overnight at 37˚C in a 6% CO_2 environment before use. The same media was used for rinsing and to fill the MSGD.

Early experiments included embryos that had been biopsied (N = 5). However, as described [16], the technique requires the embryos to be of similar shape, size, and consistency on the zona surface to be effective. The biopsy hole significantly changed the drop rates of embryos, so PGT embryos were eliminated from the present study, and the data were not included in the final analysis. A total of 21 straws were thawed, yielding 27 embryos for this study.

Embryos were thawed using commercially available media (Blastocyst Fast Freeze Thaw kit, Life Global) and following manufacturer's protocol. In brief, embryo straws were removed from liquid nitrogen storage and exposed to 30˚C water bath for thawing. Embryos were recovered from the straws, passed sequentially through 5 thaw solutions containing decreasing concentrations of cryoprotectants, and finally placed in culture medium for this study.

Once thawed, the outer diameter of the zona pellucida was estimated manually with a microscope reticle. It was also noted if the true embryo had re-expanded or remained compacted following the thawing procedure. Embryos were then dropped through the MSGD column (filled with Global medium supplemented with 20% SSS) by placing the embryo on the meniscus at the top of the drop chamber (**Figure 1**). The MSGD had been modified from the original apparatus [16] to monitor the drop chamber with a simple pen microscope (Takmly; Semarang, Indonesia), which allowed easy focus and electronic observation on a laptop computer screen. The embryo was then observed and timed as it passed through a 1 cm distance of the drop chamber, as described previously (zero-hour time point) [16]. Once measured, the embryo was recovered and placed in culture at 37˚C for a period of 3 hrs to allow full expansion of the true embryo. At three hours, the embryo was again dropped through the MSGD (3-hour time point) and returned to culture for another 21 hours (24 hours of culture total). At 24 hours, the embryos were ob-

served for evidence of hatching as a sign of continuing development in culture.

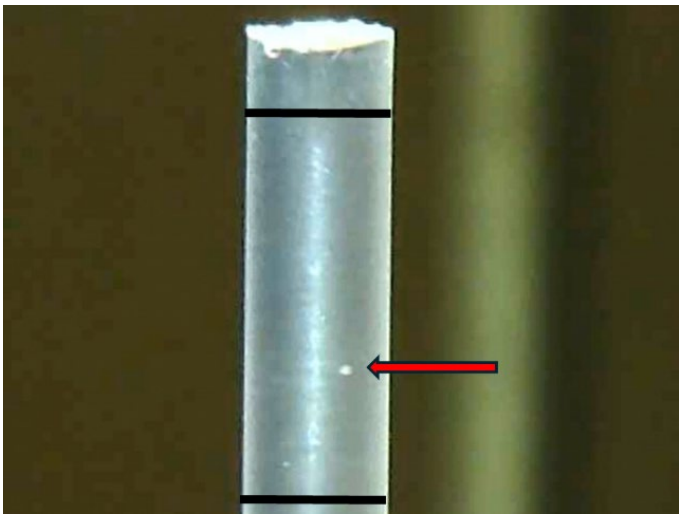


Figure 1. Demonstrates the ease of embryo visualization (red arrow) in the drop chamber as the embryo descends through one-centimeter measurement zone (black lines).

Previous animal studies had used data from a large numbers of embryos to establish drop curves for viability [14]-[18]. However, in this initial study with a limited number of human embryos available, we compared the outcome data (hatching) and initial embryo expansion (both signs of embryo viability) and compared it to the observed drop times 0 and 3-hr post thaw in an attempt to establish early validation of the technique without the need for large cohorts of embryo. Comparisons were made between the drop times of expanded and non-expanded embryos and hatched and non-hatched embryos using the student's T-test.

3. Results

As previously mentioned, and described in earlier work [14]-[18], for the system to be effective, the embryos examined must be of similar size and shape. While, there is some variability in embryo diameter, the cohorts in each group were statistically similar (**Table 1**).

Table 1. The average diameter of embryos based on which embryos re-expanded immediately after thaw, and which embryos hatched after 24 hrs in culture.

	Yes (μm)	+/− STD	No (μm)	+/− STD	P value
Expanded	169.5	+/− 17.8	164.3	+/− 33.1	0.317
Hatched	167.4	+/− 24.8	168.0	+/− 23.1	0.498

An overall initial mean descent time of 11.2 +/− 8.1 seconds was established for all twenty-seven embryos using the drop times immediately after thaw. It was found that while all but one of the embryos that continued on to hatching was at

or within one standard deviation unit of the mean (15/16; 93.8%), fully 7/11 at the 0 (63.6) embryos that failed to hatch had descent times greater than one standard deviation unit away from the mean (Figure 2). This was very similar to data from mice [16], where it was found that embryos that failed to progress tended to have descent times at the extremes of a bell-shaped curve. In contrast, the viable embryos descent times tended to cluster within one standard deviation unit above and below the mean [13] [14].

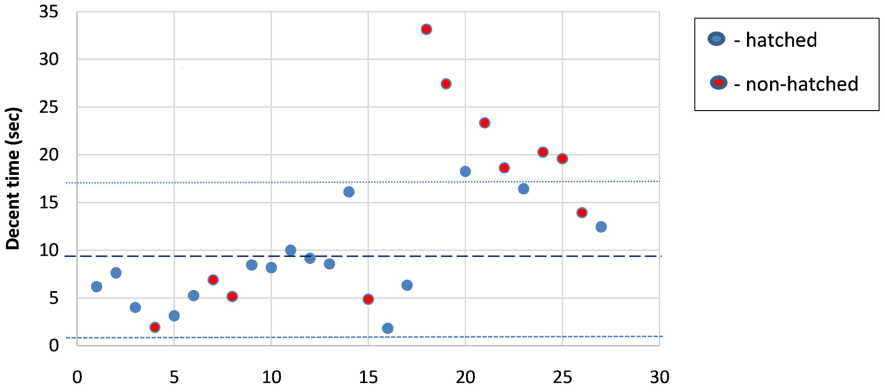


Figure 2. The relationship between embryo descent time at the 0-hr drop point through a modified specific gravity device (MSGD), and eventual survival, and development measured as embryo hatching. As in previous studies [13] [14], embryos that hatched had descent times within one standard deviation of the population mean.

The data were then re-examined to determine whether they expanded by the 3-hour time point and whether they were hatched at the 24-hour mark. As stated in the methods, embryos were dropped in MSDG chamber immediately after the thaw procedure was complete. A time when clinically, embryos may remain collapsed with no or little blastocoel cavity, but this would be a very convenient time for the clinical program to assess the viability of the embryo. Similarly, embryos were dropped in MSDG chamber at 3 hours incubation point, where clinically, it is expected that a viable embryo will show significant expansion. As expected, there was significant slowing of drop time between the 0 hr (11.2 +/− 8.1 sec) and 3 hrs (72.8 +/− 54.0 sec) drop point events ($P < 0.001$). This appeared to result from the blastocoel being filled with fluid, and nutrients from the media and the embryo's buoyancy shifting closer to that of the media in the drop chamber. This is supported by the observation that embryos scored as non-expanding demonstrated only modest increases in drop times compared to those that fully expanded (Figure 3, $P < 0.04$).

Figure 4 demonstrates the mean descent times of the embryos immediately post-thaw and after three hours of incubation to allow expansion, based on which hatched after 24 hrs of incubation versus those that failed to progress. Embryos that hatched demonstrated faster descent at both drop times ($P < 0.001$ and $P < 0.05$, respectively). While this data may appear to contradict that of the expanded embryos, based on previous research [16]. It is an expected outcome if one group,

in this case, the hatching group, has maintained better cell integrity during the freeze/thaw process. The earlier study in mice demonstrated that damaged cells leak constituents and reach equilibrium with their environment. Therefore, if a large number of cells are damaged or dead, the embryo becomes more buoyant and has increased decent times.

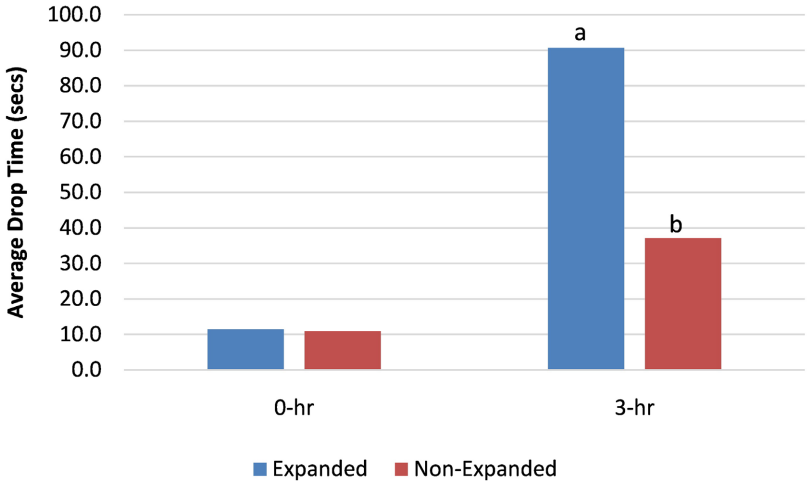


Figure 3. Correlation of the average drop times of human embryos passed through a modified specific gravity device at zero and three hours post-thaw and the corresponding post-thaw embryo expansion. This demonstrates that while there were no differences in the average drop times of embryos immediately after thaw procedures (0-hr Drop Point; $P = 0.648$), Embryos that expanded post-thaw had significantly slower descent times ($P < 0.04$) compared to embryos that did not expand after 3 hours in culture (3-hr Drop Point).

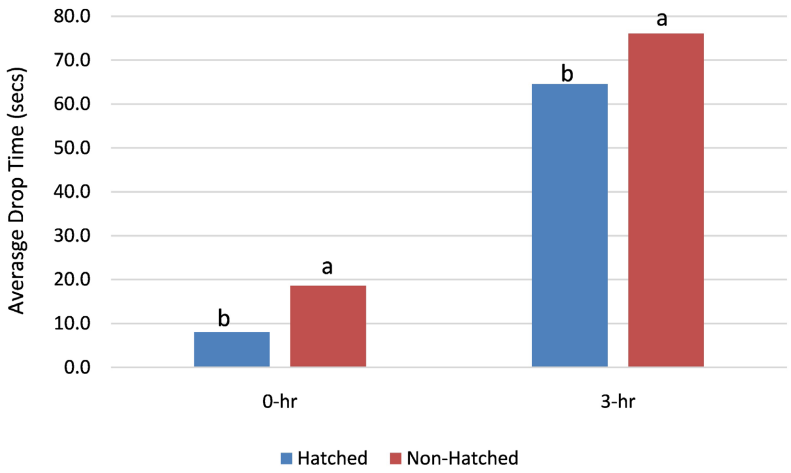


Figure 4. Correlation of the average drop times of human embryos passed through a modified specific gravity device and post-thaw embryo expansion. Embryos that hatched post-thaw demonstrated significantly faster average drop times at both the 0-hr Drop Point at the time of thawing ($P < 0.001$), and after 3 hours in culture (3-hr Drop Point) compared to embryos that failed to hatch ($P < 0.05$).

4. Discussion

While morphology has been the gold standard of embryo evaluation since the in-

ception of IVF, it is universally recognized that the system is limited. Numerous attempts have been described to provide a more robust form of embryo evaluation in an attempt to increase the overall efficiency of the process. Some, like PGT, have successfully evaluated single aspects of embryo development, such as embryo DNA integrity [7]. However, while cell-free PGT has been proposed, current technology is still based on an invasive biopsy of trophoblastic cells, and recent studies suggest such biopsies may or may not reflect the chromosomal makeup of the true embryo [20].

Recent work with morphometrics suggests a correlation between development rates and success [10]-[13] [21]. However, the usefulness of the data in a clinical setting is currently limited due to the expense of the equipment. Further, the data would reflect embryo development rather than predict future growth potential. Spent media assessment has also been suggested [12]. However, even small variations in media volume might cause a misinterpretation of embryo potential.

The current study is the first reported use of a previously described modified specific gravity technique [14]-[18] with human embryos. Data from the present study continues to suggest that a simple modified specific gravity device might be useful in predicting future embryo development. As in previous studies, the embryos with the greatest potential for development appeared to have drop times closer to the population mean rather than the extremes, as indicated by those embryos that continued to hatch. While the data from the expanded vs. non-expanded embryos might seem in conflict with the hatching data, it is not if one thinks through the physiological processes as described previously [16]. For both expansion and survival, an embryo needs to maintain membrane integrity. At thaw, cryopreserved embryos are extremely compacted from dehydration, and it is difficult for the MSGD to detect differences, as any relative differences are masked by the highly dense cellular core. However, as those embryos that have maintained membrane integrity start to expand, the density of the embryo within the zona decreases, and the descent time increases (3-hr drop point).

However, there is also a second physiological phenomenon taking place at thaw. Cells that have been damaged during the freeze-thaw process are leaking inner cytoplasmic materials. These materials are needed not only for embryo survival but also to increase the overall density of the individual cells. While fully damaged embryos will fail to expand, embryos that may expand but have suffered cellular damage will leak cytoplasmic materials, hence being less dense and taking longer to descend at both time points.

The current study is limited in both the number of embryos and the fact that, for ethical reasons, only frozen embryos were available for study. Further, based on early work (not shown), it was determined that biopsied samples could not be measured effectively. While this might seem a highly limiting factor in the use of the technique, previous studies have demonstrated that the MSGD can be performed at any stage of pre-implantation embryo development, from oocyte to blastocyst [16]. The use of the MSGD prior to biopsy might allow a complete as-

sessment of embryos for their chromosome integrity and growth potential. Coupling the MSGD with other assessment tools would provide the most complete picture of the embryos health and its potential to establish a pregnancy and for that pregnancy to progress to delivery.

Further studies are underway to both simplify and automate the MSGD, in hopes of making access to the technology easier and making future studies on a much larger scale possible in humans as already been done in other species [14]-[18]. Additionally, previous studies in other species have shown the technique can determine viability differences during earlier periods of development (zygote to morula stage; [16]. If the same is true with human embryos, the MSGD could be incorporated early in the culture period and possibly enhance or replace other invasive and non-invasive techniques in determining the best embryos for transfer.

Declaration

This work was presented in part at the 2022 Congress of the American Society of Reproductive Medicine, Anaheim, CA.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] HHS.gov (2024) Fact Sheet: *In Vitro* Fertilization (IVF) Use Across the United States.

[2] Fatemi, H., Bilger, W., Denis, D., Griesinger, G., La Marca, A., Longobardi, S., *et al.* (2021) Dose Adjustment of Follicle-Stimulating Hormone (FSH) during Ovarian Stimulation as Part of Medically-Assisted Reproduction in Clinical Studies: A Systematic Review Covering 10 Years (2007-2017). *Reproductive Biology and Endocrinology*, **19**, Article No. 68. https://doi.org/10.1186/s12958-021-00744-x

[3] Rienzi, L., Vajta, G. and Ubaldi, F. (2011) New Culture Devices in ART. *Placenta*, **32**, S248-S251. https://doi.org/10.1016/j.placenta.2011.06.018

[4] Sciorio, R. and Rinaudo, P. (2023) Culture Conditions in the IVF Laboratory: State of the ART and Possible New Directions. *Journal of Assisted Reproduction and Genetics*, **40**, 2591-2607. https://doi.org/10.1007/s10815-023-02934-5

[5] Tocariu, R., Niculae, L.E., Niculae, A.Ș., Carp-Velișcu, A. and Brătilă, E. (2024) Fresh versus Frozen Embryo Transfer in *in Vitro* Fertilization/intracytoplasmic Sperm Injection Cycles: A Systematic Review and Meta-Analysis of Neonatal Outcomes. *Medicina*, **60**, Article 1373. https://doi.org/10.3390/medicina60081373

[6] Racowsky, C., Vernon, M., Mayer, J., Ball, G.D., Behr, B., Pomeroy, K.O., *et al.* (2010) Standardization of Grading Embryo Morphology. *Journal of Assisted Reproduction and Genetics*, **27**, 437-439. https://doi.org/10.1007/s10815-010-9443-2

[7] Greco, E., Litwicka, K., Minasi, M.G., Cursio, E., Greco, P.F. and Barillari, P. (2020) Preimplantation Genetic Testing: Where We Are Today. *International Journal of Molecular Sciences*, **21**, Article 4381. https://doi.org/10.3390/ijms21124381

[8] Chavez-Badiola, A., Farías, A.F., Mendizabal-Ruiz, G., Silvestri, G., Griffin, D.K., Valencia-Murillo, R., *et al.* (2024) Use of Artificial Intelligence Embryo Selection Based

on Static Images to Predict First-Trimester Pregnancy Loss. *Reproductive BioMedicine Online*, **49**, Article 103934. https://doi.org/10.1016/j.rbmo.2024.103934

[9] Faramarzi, A., Khalili, M.A. and Omidi, M. (2017) Morphometric Analysis of Human Oocytes Using Time Lapse: Does It Predict Embryo Developmental Outcomes? *Human Fertility*, **22**, 171-176. https://doi.org/10.1080/14647273.2017.1406670

[10] Wessels, C, Penrose, L.L. and Prien, S.D. (2017) Current State of Art Embryo Selection Techniques. *Investigations in Gynecology Research & Womens Health* (*IGRWH*), **1**, 7-12. https://doi.org/10.31031/igrwh.2017.01.000502

[11] Weathers, J., Zimmerer, N., Penrose, L., Graves-Evenson, K. and Prien, S. (2013) The Relationship between Maternal Body Fat and Pre-Implantation Embryonic Weight: Implications for Survival and Long-Term Development in an Assisted Reproductive Environment. *Open Journal of Obstetrics and Gynecology*, **3**, 1-5. https://doi.org/10.4236/ojog.2013.35a2001

[12] Sciorio, R., Miranian, D. and Smith, G.D. (2022) Non-Invasive Oocyte Quality Assessment. *Biology of Reproduction*, **106**, 274-290. https://doi.org/10.1093/biolre/ioac009

[13] Kakourou, G., Mamas, T., Vrettou, C. and Traeger-Synodinos, J. (2022) An Update on Non-Invasive Approaches for Genetic Testing of the Preimplantation Embryo. *Current Genomics*, **23**, 337-352. https://doi.org/10.2174/1389202923666220927111158

[14] Pandit, S. and Sharma, R. (2022) Non Invasive Assessment of Human Oocytes and Embryos in Assisted Reproduction: Review on Present Practices and Future Trends. *Medical Journal Armed Forces India*, **78**, 7-16. https://doi.org/10.1016/j.mjafi.2021.04.002

[15] Weathers, J.D. and Prien, S.D. (2014) Estimation of Weight and Lipid Composition in Preimplantation Embryos from Jersey and Beef Breeds of Cattle. *Open Journal of Veterinary Medicine*, **4**, 261-266. https://doi.org/10.4236/ojvm.2014.411031

[16] Prien, S.D., Wessels, C.E. and Penrose, L.L. (2015) Preliminary Trials of a Specific Gravity Technique in the Determination of Early Embryo Growth Potential. *Human Reproduction*, **30**, 2076-2083. https://doi.org/10.1093/humrep/dev178

[17] Wessels, C., Penrose, L., Ahmad, K. and Prien, S. (2017) Noninvasive Embryo Assessment Technique Based on Buoyancy and Its Association with Embryo Survival after Cryopreservation. *Theriogenology*, **103**, 169-172. https://doi.org/10.1016/j.theriogenology.2017.07.010

[18] Wells, C.W., Penrose, L.L., Orth, M. and Prien, S. (2020) A Noninvasive Method for Assessing Oocyte Competency. *Case Reports in Obstetrics Gynecology and Reproductive*, **2**, 1-5. https://doi.org/10.31487/j.crogr.2020.02.02

[19] Stachecki, J.J. and Cohen, J. (2008) S3 Vitrification System: A Novel Approach to Blastocyst Freezing. *The Journal of Clinical Embryology*, **11**, 2-14.

[20] Casper, R.F. (2023) PGT-A: Houston, We Have a Problem. *Journal of Assisted Reproduction and Genetics*, **40**, 2325-2332. https://doi.org/10.1007/s10815-023-02913-w

[21] Du, R., Zhao, D., Kang, K., Wang, F., Xu, R., Chi, C., *et al.* (2022) A Review of Pre-Implantation Genetic Testing Technologies and Applications. *Reproductive and Developmental Medicine*, **7**, 20-31. https://doi.org/10.1097/rd9.0000000000000049