Oocyte vitrification for fertility preservation is an evolving practice requiring a new mindset: societal, technical, clinical, and basic science-driven evolutions

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Infertility is a condition with profound social implications. Indeed, it is not surprising that evolutions in both medicine and society affect the way in vitro fertilization is practiced. The keywords in modern medicine are the four principles, which implicitly involve a constant update of our knowledge and our technologies to fulfill the "prediction" and "personalization" tasks, and a continuous reshaping of our mindset in view of all relevant societal changes to fulfill the "prevention" and "participation" tasks. A worldwide aging population whose life priorities are changing requires that we invest in fertility education, spreading actionable information to allow women and men to make meaningful reproductive choices. Fertility preservation for both medical and nonmedical reasons is still very much overlooked in many countries worldwide, demanding a comprehensive update of our approach, starting from academia and in vitro fertilization laboratories, passing through medical offices, and reaching out to social media. Reproduction medicine should evolve from being a clinical practice to treat a condition to being a holistic approach to guarantee patients' reproductive health and well-being. Oocyte vitrification for fertility preservation is the perfect use case for this transition. This tool is acquiring a new identity to comply with novel indications and social needs, persisting technical challenges, brand-new clinical technologies, and novel revolutions coming from academia. This "views and reviews" piece aims at outlining the advancement of oocyte vitrification from all these tightly connected perspectives. (Fertil Steril® 2024;■:■–■. ©2024 by American Society for Reproductive Medicine.)
Key Words: Oocyte vitrification, fertility preservation, oocyte quality, oocyte competence

WOMAN HEALTH AND WELL-BEING: FERTILITY PRESERVATION AS PART OF A HOLISTIC APPROACH

Daniela Galliano, M.D. Ph.D.

Although the 20th century was characterized by population growth, the 21st century is marked by population aging. Therefore, there is an increased interest in rejuvenation, the prevention or delaying of aging, and its detrimental effects on our cells, driven by recently proposed hallmarks (1). The number of centers offering well-being treatments, including vitamins, diets, exercise, and a healthy lifestyle, is proliferating at a rapid pace. In parallel, women are demanding more integrated and holistic solutions for personal care. In the coming years, women-centric healthcare may apply preventive personalized measures from puberty to old age, even specific to women affected by preexisting diseases. Fertility preservation plays a key role in this comprehensive approach. Tissues and organs start to be unable to repair DNA damage after 40–45 years of age, yet they retain their activity over several decades. However, the ovary has an abrupt and definite loss of function at this age, affecting the woman's life, health, and well-being when motherhood has not yet been achieved. To be able to act on the biologic clock of the ovary and of the whole reproductive system according to individual planning is of paramount importance for women. Furthermore, its correct functioning and the possibility to embrace motherhood, when desired, will always play a pivotal role in a woman's life. To our knowledge, oocyte vitrification at an

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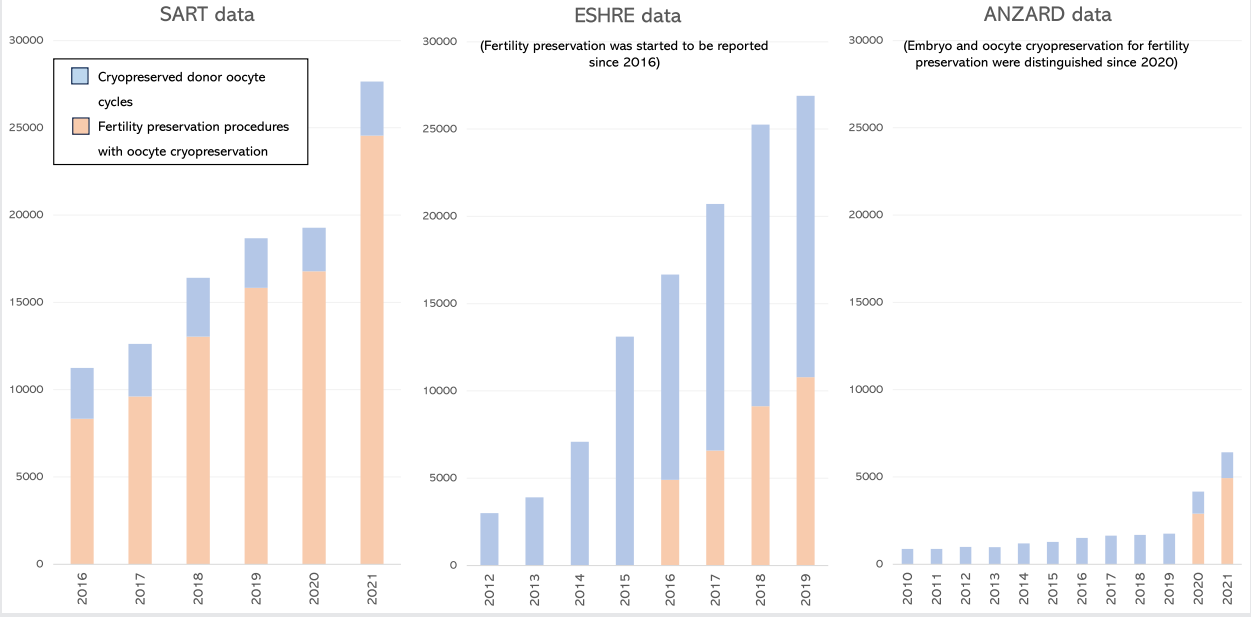
appropriate age is functional for gaining personal freedom and emancipation. The field is evolving, and we are still missing some evidence-based data to educate and counsel our patients. However, some facts are already evident, such as that fertility preservation can be opted for by healthy women but also by women suffering from diseases affecting their ovarian reserve, like cancer (2, 3) or endometriosis (4, 5). When cumulative live birth rates are analyzed, age is the main variable affecting the outcomes (6). Thus, efforts must be directed toward reducing the age of oocyte cryopreservation by urgently sparking more information, education, and awareness. All women must be well informed about the process of ovarian aging and be familiar with predictive factors of ovarian reserve, such as serum antimüllerian hormone levels and antral follicle count, to adequately plan and empower themselves with informed reproductive decisions while promoting gender equality and ensuring emotional well-being. Clinicians must also avoid the narrative that oocyte vitrification is insurance for future fertility and stress the concept of increased chances of conceiving a genetically related child. Because society is constantly evolving, it remains essential that fertility preservation is comprehended and upheld from a holistic and societal perspective, ensuring that women make informed reproductive decisions while safeguarding their mental and emotional well-being and avoiding societal pressures and stigmatization.

STANDARDIZATION AND CONSISTENCY OF OOCYTE VITRIFICATION: THERE IS STILL ROOM FOR IMPROVEMENT

Anabella Marconetto, M.Sc., and Laura Rienzi, Prof.

Because the American Society for Reproductive Medicine (ASRM) removed the "experimental" label from oocyte vitrification in 2013, the demand for this technique has increased dramatically worldwide (7–9). In the following years, it has been consistently shown to be reproducible, safe, and cost-effective, until it became the gold standard approach for fertility preservation (10–16). Worldwide registry data show a continuous increase in oocyte cryopreservation implementation for both egg donation and fertility preservation (Fig. 1). However, the clinical outcomes are variable across countries and clinics. Indeed, to date, vitrification is mostly conducted manually, thus requiring well-trained, constantly monitored, and experienced operators. Variations to the protocols exist across laboratories and even between operators, inevitably affecting their performance, as testified by the variability in the cryo-survival rates retrievable from the literature (2, 17–20). Strict quality-controls are therefore essential, that include tracking of the learning curves among trainees and undertaking a continuous monitoring of individual performance and protocol agreement (17, 21, 22). In view of this, it is not surprising

FIGURE 1



Data about oocyte cryopreservation for fertility preservation and oocyte donation from SART, ESHRE and ANZARD registries. The data show the sharp and constant increase in the adoption of this technique throughout the last decade. SART data were retrieved from https://sartcorsonline.com/CSR/PublicSnapshotReport?ClinicPKID=0&reportingYear=2020 for all years; ESHRE data were retrieved from https://www.eshre.eu/Data-collection-and-research/Consortia/EIM/Publications.aspx; ANZARD data were retrieved from https://npesu.unsw.edu.au/data-collection/australian-new-zealand-assisted-reproduction-database-anzard. ESHRE data are "provided by either national registries or registries based on initiatives of medical associations and scientific organizations or committed persons in one of the 44 countries." LBR = live birth rate; ET = embryo transfer; PR = pregnancy rate.

Cimadomo. Oocyte vitrification for fertility preservation. Fertil Steril 2024.

that automation emerged as a promising strategy to standardize (oocyte) vitrification. Semiautomated devices were reported as efficient as the operators (23, 24), yet appropriate validation is still required before clinical implementation, as higher procedural timings and less staff convenience were, for instance, reported in a recent German randomized controlled trial (25). After years of limited evolution in this field, lately a faster oocyte vitrification protocol has been suggested (26), and Liebermann et al. (27) reported promising results with a novel and faster protocol for blastocyst warming. Ultrafast oocyte vitrification and warming are the evident next steps. When confirmed, these advances might improve the reproducibility of the technique, although they also involve a more efficient and time-saving workflow in the in vitro fertilization (IVF) laboratory. Perhaps because of the variability in the outcomes across centers, oocyte vitrification has been constantly challenged throughout years, and this quest for optimization triggered disruptive ideas questioning the basics, overthrowing dogmas, and finally resulting in breakthroughs for IVF treatment. A new era of (oocyte) vitrification is about to come, and fertility preservation is the most pressing social need, as clearly testified by its average 25%- and 30%-increase per year since 2016 according to the Society for Assisted Reproductive Technology and European Society of Human Reproduction and Embryology data, respectively, a figure that reached up to a 46%- and 70%-increase in the biennium 2020–2021 in the USA and Australia–New Zealand, respectively (Fig. 1).

THE ONGOING EVOLUTION OF OOCYTE EVALUATION: FROM QUANTITY TO QUANTIFICATION OF QUALITATIVE ASSESSMENTS

Danilo Cimadomo, Ph.D., and Ana Cobo, Ph.D.

Fertility preservation success depends on technical, clinical, and patient intrinsic factors. Understanding the role played by all contributing features is mandatory to comply with the highest possible standards and provide trustworthy information, empowering women in their reproductive decision-making process. The number of oocytes retrieved and maternal age at oocyte retrieval is crucial predictors of fertility preservation outcome (2, 28). Both factors are closely connected, as the progressive decrease of the ovarian reserve has a direct impact on both oocytes' cryo-survival and competence. Elective fertility preservation in women younger than 35 years involves cumulative probabilities of live birth ranging from 30% to 45% when 8–10 oocytes are vitrified, and each additional oocyte cryopreserved has an enormous value to the point that a 70% chance can be achieved with 15 oocytes and 95% with 25 (2). Beyond the 35-year-old threshold, the number of oocytes required is clearly larger, making the task of fertility preservation much more challenging. Strategies exist to increase the number of oocytes collected in a short timeframe, including, for instance, egg banking, DuoStim, random start from the clinical perspective (29–33), and rescue-in vitro maturation (IVM) from the laboratory perspective. This practice of rescuing immature oocytes for clinical use, although still controversial in standard intracytoplasmic sperm injection cycles and discouraged in oocyte donation programs, is instead generally accepted for fertility preservation purpose. Indeed, although germinal vesicle and metaphase-I oocytes maturing in vitro to the metaphase-II (MII) stage suffer from reduced developmental competence to blastocysts, they can still represent a chance that might be relevant to certain categories of poor prognosis patients, including women undergoing fertility preservation (34, 35). Indeed, the rescue-IVM approach has been dusted off lately after being overlooked for years. Nevertheless, standard operating procedures, basic research, and postnatal follow-up are still required before they can be safely implemented in routine clinical practice.

Beyond oocyte quantity, vitrification protocol safety, and operators' technical skills, another key aspect to predicting fertility preservation success is a definition of oocyte "quality" that should be clinically relevant but instead is very difficult to standardize (36). The quality of the female gametes retrieved after ovarian stimulation is the result of an intricate process of maturation within the follicle microenvironment (37). Once in the IVF clinic, microscopic assessment is the most immediate and widely used approach to estimating its developmental potential. However, an ideal morphology (i.e., a spherical structure enclosed by a uniform zona pellucida, with a homogeneous translucent cytoplasm free of inclusions and with a size-appropriate polar body [38, 39]) characterizes only approximately 30% of the oocytes, and the predictive power of all morphological abnormalities (e.g., the presence of smooth endoplasmic reticulum aggregates, vacuoles, refractile bodies, volume, shape, granularity, first polar body anomalies, and others) on their competence is largely limited. Indeed, two systematic reviews, respectively summarizing 50 and 76 studies, revealed highly conflicting results and generally modest clinical implications for oocytes' morphological abnormalities, with the only exception of giant oocytes, whose use was discouraged because of a considerable risk of ploidy abnormalities (40, 41). Moreover, oocyte dimorphisms did not seem to affect cryo-survival rates after warming and yield comparable results to morphologically normal oocytes (42). Nonetheless, the recent introduction of artificial intelligence (AI)-powered tools in IVF treatment put oocyte morphological assessment under the spotlight again (43, 44), as it might involve the production of morphometric, measurable, reproducible, and more objective information to standardize this practice across clinics (45). To counteract the lack of an efficient visual scoring system, the most immediate application of AI related to oocytes' assessment is fertility preservation, because at present, women cryopreserving their gametes do not receive any feedback regarding their quality. Prospective multicenter investigations of clinical reliability are now required to unveil the true power of these evaluations, which are otherwise limited to being an intriguing use case grounded on no hard data. Along with AI-powered morphological assessments, genomic and exomic data are accumulating that might involve a more objective estimation of intrinsic oocytes' competence in the preconceptional period (46). For instance, whole exome sequencing in women subject to recurrent oocyte maturation defects and/or preimplantation embryo lethality after consecutive IVF cycles is helping unveil pathogenic variants associated with infertile endophenotypes, such as recurrent low oocyte maturation rate, low fertilization rate, and

preimplantation developmental arrest (47, 48). In the future, this information might be critical to justify results consistently lower than expected in poor prognosis patients. Yet, whole exome sequencing may find a larger application in the preconceptional period to guide reproductive decision-making, especially concerning fertility preservation. Although oocyte noninvasive screening of follicular fluids and cumulus cells has resulted in limited clinical utility to date, novel comprehensive omic analyses integrated with more objective morphological assessments might help determine new biomarkers of oocyte and/or early embryo developmental incompetence in the coming years (49). Until these promising perspectives provide us with powerful clinical tools, the age-adjusted number of oocytes cryopreserved still prevails on any qualitative assessment in the prediction of fertility preservation outcomes (6) (Fig. 2 summarizes the content of this paragraph).

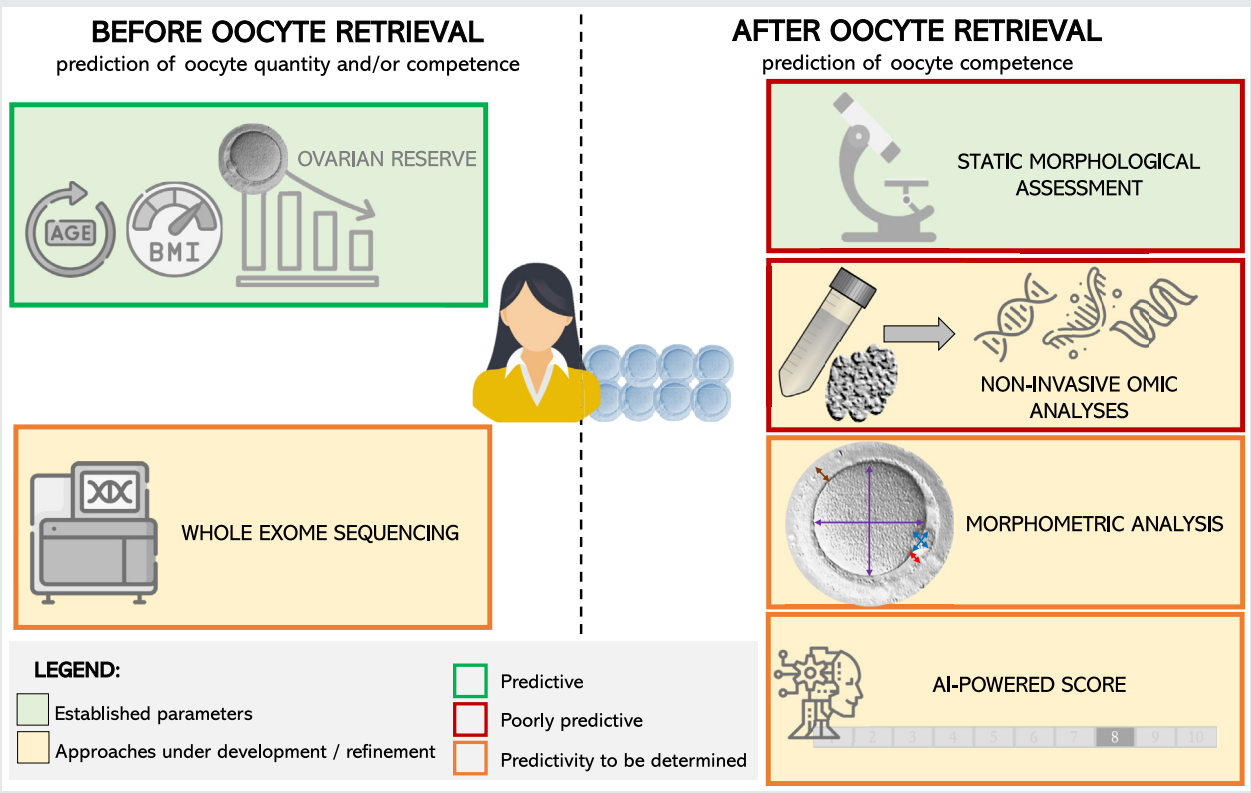
PREDICTION OR IMPROVEMENT OF OOCYTE COMPETENCE: FUTURE PERSPECTIVES FROM BASIC SCIENCE AND ANIMAL MODELS

Giulia Fiorentino, Ph.D., and Maurizio Zuccotti, Prof.

Basic research in animal models improves our understanding of the role of specific molecules in the regulation of oocytes' growth during folliculogenesis, but it also allows the design of approaches aimed at enhancing gametes' developmental competence. The two main approaches envisage either the supplementation of IVM media with oocyte-secreted factors, cytokines, or modulators of the sirtuin (SIRT) signaling pathway, among others, or advanced gametes' micromanipulation. Among the many studies in the literature, we selected and briefly summarized some with reasonable translational potential.

A recent article demonstrated that murine oocytes' mitochondrial activity and developmental competence to blastocyst might be boosted with pre-IVM 2-hour culture in a medium supplemented with C-type natriuretic peptide, estradiol, follicle-stimulating hormone, and bone morphogenetic protein-15 (50, 51). Similarly, another study reported that the addition of IVM medium with L-cysteine, a bone morphogenetic protein-15 and growth and differentiation factor-9 activator, augmented bovine oocytes' developmental competence (52). In addition, supplementation with different combinations of cytokines, namely fibroblast growth factor-2, leukemia inhibitory factor plus insulin-like growth factor 1, or epidermal growth factor plus insulin-like growth factor 1, respectively, enhanced pig (53) and guinea pig (54) oocytes' nuclear maturation and significantly increased their

FIGURE 2



Summary of the current and future parameters and approaches available to predict oocyte quantity and/or quality before and after oocyte retrieval for fertility preservation.

Cimadomo. Oocyte vitrification for fertility preservation. Fertil Steril 2024.

blastocyst development. Rapamycin is another molecule that, when added to IVM medium, contributed to the better maturation and developmental competence of bovine (55), murine (56), and porcine (57) oocytes. Antioxidants also provided promising evidence when IVM was conducted in animal models. The SIRT protein family, for instance, emerged as a regulator of oocyte aging and chromosomal stability (58–60), and because of its interaction with reactive oxygen species, it was directly involved in protecting oocytes from oxidative stress. To date, the most extensively tested IVM supplement is melatonin, which has a clear modulating effect on the SIRT pathway (61–63), especially evident because it involves a milder quality decline during oocyte maturation and embryonic development in aged mice (64). Furthermore, when combined with vitamin C, melatonin led to a significant reduction in reactive oxygen species and, as a result, enhanced blastocyst development in mouse (65) and sheep (66) oocytes. More recently, the supplementation of the IVM medium with spermidine (67) was also shown to improve both oocytes' maturation and embryo development in aged mice and in pigs subject to oxidative stress via enhanced mitochondrial activity (68).

At a more preclinical level, a few studies suggested that direct micromanipulations of female gametes might result in cytoplasmic rescue (69). For instance, maternal spindle transfer of either in vitro-aged (70) or subfertile (71) mouse MII oocytes into the cytoplasm of good-quality gametes reinstated the developmental potential of blastocysts by restoring the function of cytoplasmic factors involved in cytokinesis.

This brief overview of numerous studies on the basis of model animals testifies how active and promising the research is in this field; a future where oocytes' maturation and developmental competence might be improved in vitro, ahead of their vitrification, can be more realistically envisaged in the coming years.

CONCLUSIONS

The need for IVF treatments is constantly growing worldwide, in parallel with the adoption of oocyte vitrification for fertility preservation. Meanwhile, the implementation of technological advances, such as AI-based gamete assessment and automation, promises a more reliable prediction of oocyte competence and a higher standardization of vitrification protocols and performance in the coming years. Lately, long-lasting vitrification and warming protocols have been put under the spotlight again in view of forthcoming evolutions, whereas basic science investigations, although mostly coming from animal models, foresee a future where the acquisition of oocyte competence could be modulated during the germinal vesicle to MII transition and/or boosted via advanced micromanipulation approaches in vitro.

At present, the theme of oocyte vitrification for fertility preservation shall no longer be perceived as a taboo but as a tool to safeguard women's reproductive autonomy. The societal implications of the condition of infertility demand more education. In this context, IVF practitioners must be able to communicate efficiently and design a multidisciplinary team to take care of each woman's health and well-being through a holistic approach. We have the opportunity to enhance oocyte vitrification for fertility preservation from societal, technical, and clinical perspectives, and it is our duty not to waste this opportunity.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Laura Rienzi: Conceptualization, Data curation, Writing - original draft, Writing - review & editing. **Danilo Cimadomo:** Conceptualization, Data curation, Writing - original draft, Writing - review & editing. **Anabella Marconetto:** Data curation, Writing - original draft. **Daniela Galliano:** Writing - original draft. **Ana Cobo:** Writing - original draft. **Maurizio Zuccotti:** Writing - original draft. **Giulia Fiorentino:** Writing - original draft.

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REFERENCES

1. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: an expanding universe. Cell 2023;186:243–78.
2. Cobo A, García-Velasco J, Domingo J, Pellicer A, Remohí J. Elective and Onco-fertility preservation: factors related to IVF outcomes. Hum Reprod 2018;33:2222–31.
3. Condorelli M, Demeestere I. Challenges of fertility preservation in non-oncological diseases. Acta Obstet Gynecol Scand 2019;98:638–46.
4. Streuli I, Benard J, Hugon-Rodin J, Chapron C, Santulli P, Pluchino N. Shedding light on the fertility preservation debate in women with endometriosis: a swot analysis. Eur J Obstet Gynecol Reprod Biol 2018;229:172–8.
5. Cobo A, Giles J, Paolelli S, Pellicer A, Remohí J, García-Velasco JA. Oocyte vitrification for fertility preservation in women with endometriosis: an observational study. Fertil Steril 2020;113:836–44.
6. Cobo A, García-Velasco JA, Remohí J, Pellicer A. Oocyte vitrification for fertility preservation for both medical and nonmedical reasons. Fertil Steril 2021;115:1091–101.
7. Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. Mature oocyte cryopreservation: a guideline. Fertil Steril 2013;99:37–43.
8. Johnston M, Richings NM, Leung A, Sakkas D, Catt S. A major increase in oocyte cryopreservation cycles in the USA, Australia and New Zealand since 2010 is highlighted by younger women but a need for standardized data collection. Hum Reprod 2021;36:624–35.
9. Kawwass JF, Crawford S, Hipp HS. Frozen eggs: national autologous oocyte thaw outcomes. Fertil Steril 2021;116:1077–84.
10. Chamayou S, Sicali M, Alecci C, Ragolia C, Liprino A, Nibali D, et al. The accumulation of vitrified oocytes is a strategy to increase the number of euploid available blastocysts for transfer after preimplantation genetic testing. J Assist Reprod Genet 2017;34:479–86.
11. Forman EJ, Li X, Ferry KM, Scott K, Treff NR, Scott RT Jr. Oocyte vitrification does not increase the risk of embryonic aneuploidy or diminish the implantation potential of blastocysts created after intracytoplasmic sperm injection: a novel, paired randomized controlled trial using DNA fingerprinting. Fertil Steril 2012;98:644–9.
12. Cobo A, Serra V, Garrido N, Olmo I, Pellicer A, Remohí J. Obstetric and perinatal outcome of babies born from vitrified oocytes. Fertil Steril 2014;102:1006–15.e4.
13. Noyes N, Porcu E, Borini A. Over 900 oocyte cryopreservation babies born with no apparent increase in congenital anomalies. Reprod Biomed Online 2009;18:769–76.
14. Levi-Setti PE, Borini A, Patrizio P, Bolli S, Vigiliano V, De Luca R, et al. ART results with frozen oocytes: data from the Italian ART registry (2005-2013). J Assist Reprod Genet 2016;33:123–8.

15. Ethics Committee of the American Society for Reproductive Medicine. Planned oocyte cryopreservation for women seeking to preserve future reproductive potential: an Ethics Committee opinion. Fertil Steril 2018;110:1022–8.
16. Di Pietro C, Vento M, Guglielmino MR, Borzì P, Santonocito M, Ragusa M, et al. Molecular profiling of human oocytes after vitrification strongly suggests that they are biologically comparable with freshly isolated gametes. Fertil Steril 2010;94:2804–7.
17. Maggiulli R, Vaiarelli A, Cimadomo D, Giancani A, Tacconi L, Fabozzi G, et al. Fertility preservation through oocyte vitrification: clinical and laboratory perspectives. J Vis Exp 2021;(175).
18. Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod 2010;25:66–73.
19. Cornet-Bartolomé D, Rodriguez A, García D, Barragán M, Vassena R. Efficiency and efficacy of vitrification in 35 654 sibling oocytes from donation cycles. Hum Reprod 2020;35:2262–71.
20. Wennberg AL, Schildauer K, Brännström M. Elective oocyte freezing for nonmedical reasons: a 6-year report on utilization and in vitro fertilization results from a Swedish center. Acta Obstet Gynecol Scand 2019;98:1429–34.
21. Vajta G, Nagy ZP. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. Reprod Biomed Online 2006;12:779–96.
22. Cobo A, Garrido N, Pellicer A, Remohí J. Six years' experience in ovum donation using vitrified oocytes: report of cumulative outcomes, impact of storage time, and development of a predictive model for oocyte survival rate. Fertil Steril 2015;104:1426–34.e1.
23. Miwa A, Noguchi Y, Hosoya K, Mori Y, Sato T, Kasahara Y, et al. Equivalent clinical outcome after vitrified-thawed blastocyst transfer using semi-automated embryo vitrification system compared with manual vitrification method. Reprod Med Biol 2020;19:164–70.
24. Barberet J, Ducreux B, Bruno C, Guilleman M, Simonot R, Lieury N, et al. Comparison of oocyte vitrification using a semi-automated or a manual closed system in human siblings: survival and transcriptomic analyses. J Ovarian Res 2022;15:128.
25. Hajek J, Baron R, Sandi-Monroy N, Schansker S, Schoepper B, Depenbusch M, et al. A randomised, multi-center, open trial comparing a semi-automated closed vitrification system with a manual open system in women undergoing IVF. Hum Reprod 2021;36:2101–10.
26. Gallardo M, Saenz J, Risco R. Human oocytes and zygotes are ready for ultrafast vitrification after 2 minutes of exposure to standard CPA solutions. Sci Rep 2019;9:15986.
27. Liebermann J, Hrvojevic K, Hirshfeld-Cytron J, Brohammer R, Wagner Y, Susralski A, et al. Fast and furious: pregnancy outcome with one-step rehydration in the warming protocol for human blastocysts. Reprod Biomed Online. In press.
28. Doyle JO, Richter KS, Lim J, Stillman RJ, Graham JR, Tucker MJ. Successful elective and medically indicated oocyte vitrification and warming for autologous in vitro fertilization, with predicted birth probabilities for fertility preservation according to number of cryopreserved oocytes and age at retrieval. Fertil Steril 2016;105:459–66.e2.
29. Tsampras N, Gould D, Fitzgerald CT. Double ovarian stimulation (DuoStim) protocol for fertility preservation in female oncology patients. Hum Fertil (Camb) 2017;20:248–53.
30. Vaiarelli A, Cimadomo D, Petriglia C, Conforti A, Alviggi C, Ubaldi N, et al. DuoStim – a reproducible strategy to obtain more oocytes and competent embryos in a short time-frame aimed at fertility preservation and IVF purposes. A systematic review. Ups J Med Sci 2020;125:121–30.
31. Alteri A, Pisaturo V, Nogueira D, D'Angelo A. Elective egg freezing without medical indications. Acta Obstet Gynecol Scand 2019;98:647–52.
32. Cakmak H, Katz A, Cedars MI, Rosen MP. Effective method for emergency fertility preservation: random-start controlled ovarian stimulation. Fertil Steril 2013;100:1673–80.
33. Cakmak H, Rosen MP. Random-start ovarian stimulation in patients with cancer. Curr Opin Obstet Gynecol 2015;27:215–21.
34. Elkhatib I, Nogueira D, Bayram A, Abdala A, Del Gallego R, Melado L, et al. How to identify patients who would benefit from delayed-matured oocytes insemination: a sibling oocyte and ploidy outcome study. Hum Reprod 2023;38:1473–83.
35. Mandelbaum RS, Awadalla MS, Smith MB, Violette CJ, Klooster BL, Danis RB, et al. Developmental potential of immature human oocytes aspirated after controlled ovarian stimulation. J Assist Reprod Genet 2021;38:2291–9.
36. Chang CC, Shapiro DB, Nagy ZP. The effects of vitrification on oocyte quality. Biol Reprod 2022;106:316–27.
37. Conti M, Franciosi F. Acquisition of oocyte competence to develop as an embryo: integrated nuclear and cytoplasmic events. Hum Reprod Update 2018;24:245–66.
38. ALPHA Scientists In Reproductive Medicine, ESHRE Special Interest Group Embryology. Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Proceedings of the an expert meeting. Reprod Biomed Online. Istanbul consensus workshop on embryo assessment 2011;22:632–46.
39. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Proceedings of the an expert meeting. Hum Reprod. The Istanbul consensus workshop on embryo assessment 2011;26:1270–83.
40. Rienzi L, Vajta G, Ubaldi F. Predictive value of oocyte morphology in human IVF: a systematic review of the literature. Hum Reprod Update 2011;17:34–45.
41. Bartolacci A, Intra G, Coticchio G, dell'Aquila M, Patria G, Borini A. Does morphological assessment predict oocyte developmental competence? A systematic review and proposed score. J Assist Reprod Genet 2022;39:3–17.
42. Coello A, Sanchez E, Vallejo B, Meseguer M, Remohí J, Cobo A. Effect of oocyte morphology on post-warming survival and embryo development in vitrified autologous oocytes. Reprod Biomed Online 2019;38:313–20.
43. Letort G, Eichmuller A, Da Silva C, Nikalayevich E, Crozet F, Salle J, et al. An interpretable and versatile machine learning approach for oocyte phenotyping. J Cell Sci 2022;135:jcs260281.
44. Targosz A, Przystałka P, Wiaderkiewicz R, Mrugacz G. Semantic segmentation of human oocyte images using deep neural networks. Biomed Eng Online 2021;20:40.
45. Bassil R, Casper RF, Meriano J, Smith R, Haas J, Mehta C, et al. Can oocyte diameter predict embryo quality? Reprod Sci 2021;28:904–8.
46. Capalbo A, Poli M, Riera-Escamilla A, Shukla V, Kudo Høffding M, Krausz C, et al. Preconception genome medicine: current state and future perspectives to improve infertility diagnosis and reproductive and health outcomes based on individual genomic data. Hum Reprod Update 2021;27:254–79.
47. Capalbo A, Buonaiuto S, Figliuzzi M, Damaggio G, Girardi L, Caroselli S, et al. Maternal exome analysis for the diagnosis of oocyte maturation defects and early embryonic developmental arrest. Reprod Biomed Online 2022;45:508–18.
48. Innocenti F, Fiorentino G, Cimadomo D, Soscia D, Garagna S, Rienzi L, et al. Maternal effect factors that contribute to oocytes developmental competence: an update. J Assist Reprod Genet 2022;39:861–71.
49. Sciorio R, Miranian D, Smith GD. Non-invasive oocyte quality assessment. Biol Reprod 2022;106:274–90.
50. Santiquet NW, Greene AF, Becker J, Barfield JP, Schoolcraft WB, Krisher RL. A pre-in vitro maturation medium containing cumulus oocyte complex ligand-receptor signaling molecules maintains meiotic arrest, supports the cumulus oocyte complex and improves oocyte developmental competence. Mol Hum Reprod 2017;23:594–606.
51. Zeng HT, Ren Z, Guzman L, Wang X, Sutton-McDowall ML, Ritter LJ, et al. Heparin and cAMP modulators interact during pre-in vitro maturation to affect mouse and human oocyte meiosis and developmental competence. Hum Reprod 2013;28:1536–45.
52. Elgebaly MM, Hazaa ABM, Amer HA, Mesalam A. L-cysteine improves bovine oocyte developmental competence in vitro via activation of oocyte-derived growth factors BMP-15 and GDF-9. Reprod Domest Anim 2022;57:734–42.
53. Yuan Y, Spate LD, Redel BK, Tian Y, Zhou J, Prather RS, et al. Quadrupling efficiency in production of genetically modified pigs through improved oocyte maturation. Proc Natl Acad Sci U S A 2017;114:E5796–804.
54. Cañón-Beltrán K, García-García RM, Cajas YN, Fierro N, Lorenzo PL, Arias-Álvarez M. Improvement of oocyte competence and in vitro oocyte

maturation with EGF and IGF-I in guinea pig model. Theriogenology 2024; 214:206–14.
55. Khatun H, Islam MR, Khan M, Sultana F. Supplementation of rapamycin during in vitro maturation promotes oocyte quality and subsequent embryo development in bovine. Bull Natl Res Cent 2023;47:93.
56. Yang Q, Xi Q, Wang M, Long R, Hu J, Li Z, et al. Rapamycin improves the quality and developmental competence of mice oocytes by promoting DNA damage repair during in vitro maturation. Reprod Biol Endocrinol 2022;20:67.
57. Park HJ, Heo GD, Yang SG, Koo DB. Rapamycin encourages the maintenance of mitochondrial dynamic balance and mitophagy activity for improving developmental competence of blastocysts in porcine embryos in vitro. Mol Reprod Dev 2023;90:236–47.
58. Vazquez BN, Vaquero A, Schindler K. Sirtuins in female meiosis and in reproductive longevity. Mol Reprod Dev 2020;87:1175–87.
59. Vo KCT, Sato Y, Kawamura K. Improvement of oocyte quality through the SIRT signaling pathway. Reprod Med Biol 2023;22:e12510.
60. Pollard CL, Gibb Z, Swegen A, Grupen CG. NAD+, Sirtuins and PARPs: enhancing oocyte developmental competence. J Reprod Dev 2022;68:345–54.
61. Guo Y, Sun J, Bu S, Li B, Zhang Q, Wang Q, et al. Melatonin protects against chronic stress-induced oxidative meiotic defects in mice MII oocytes by regulating SIRT1. Cell Cycle 2020;19:1677–95.
62. Li C, He X, Huang Z, Han L, Wu X, Li L, et al. Melatonin ameliorates the advanced maternal age-associated meiotic defects in oocytes through the SIRT2-dependent H4K16 deacetylation pathway. Aging (Albany, NY) 2020;12:1610–23.
63. Song C, Peng W, Yin S, Zhao J, Fu B, Zhang J, et al. Melatonin improves age-induced fertility decline and attenuates ovarian mitochondrial oxidative stress in mice. Sci Rep 2016;6:35165.
64. Qu J, Hu H, Niu H, Sun X, Li Y. Melatonin restores the declining maturation quality and early embryonic development of oocytes in aged mice. Theriogenology 2023;210:110–8.
65. Navid S, Saadatian Z, Talebi A. Assessment of developmental rate of mouse embryos yielded from in vitro fertilization of the oocyte with treatment of melatonin and vitamin C simultaneously. BMC Womens Health 2023;23:525.
66. Tripathi SK, Nandi S, Gupta PSP, Mondal S. Antioxidants supplementation improves the quality of in vitro produced ovine embryos with amendments in key development gene expressions. Theriogenology 2023;201:41–52.
67. Kramer DL, Diegelman P, Jell J, Vujcic S, Merali S, Porter CW. Polyamine acetylation modulates polyamine metabolic flux, a prelude to broader metabolic consequences. J Biol Chem 2008;283:4241–51.
68. Zhang Y, Bai J, Cui Z, Li Y, Gao Q, Miao Y, et al. Polyamine metabolite spermidine rejuvenates oocyte quality by enhancing mitophagy during female reproductive aging. Nat Aging 2023;3:1372–86.
69. Reader KL, Stanton JL, Juengel JL. The role of oocyte organelles in determining developmental competence. Biology (Basel) 2017;6:35.
70. Yamada M, Egli D. Genome transfer prevents fragmentation and restores developmental potential of developmentally compromised postovulatory aged mouse oocytes. Stem Cell Rep 2017;8:576–88.
71. Costa-Borges N, Spath K, Miguel-Escalada I, Mestres E, Balmaseda R, Serafín A, et al. Maternal spindle transfer overcomes embryo developmental arrest caused by ooplasmic defects in mice. eLife 2020;9:E48591.