Backed by Science: **Ultra-Fast Solutions by Kitazato**

A scientific overview of key studies about Vitrification & Warming Protocols Outcomes

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This whitepaper compiles key scientific findings from studies presented at the 41st ESHRE Annual Meeting (Paris, 2025) and other peer-reviewed publications evaluating Ultra-Fast Vitrification and Warming protocols.

These protocols have been conducted using Kitazato's Ultra-Fast Solutions and protocols, specifically designed to streamline cryopreservation procedures. **The aim is to summarize and contextualize clinical and laboratory outcomes comparing Ultra-Fast versus conventional protocols.**

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Oral Communications presented in 41st ESHRE Annual Meeting at Paris (France), 2025

Optimizing morphokinetic embryo development: oocyte and embryo vitrification and thawing via ultra-rapid warming protocol.

Kotliarova, O., Aydin, B., Dorofeyeva, U., Hudkova, D., Pylypenko, N., Vitushynska, M., Misyura, Y., Rozhak, N., Unsal, E., Kal, N. S., Kubaskova, T. M., & Babarikova, V.

The study was conducted in collaboration with several fertility and genetic clinics across Europe, including: Ivf Georgia (Tbilisi, Georgia), Ovogene Ivf clinics in Bratislava and Kosice (Slovakia), Art Clinic Ivf (Lviv, Ukraine), Mikrogen Genetic Clinic (Ankara, Turkey), Stem and Gene ART Clinic (Tirana, Albania), and SPLN Sanatórium pre liečbu neplodnosti Ivf (Kosice, Slovakia).

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Study Design
Prospective multicenter cohort (17 IVF clinics), including 1165 oocytes vitrified via ultra-rapid protocol (1 min ES / 1 min VS) for 117 recipients (2023–2024).

Groups
Standard warming (Group A, n=634) vs. ultra-rapid warming (Group B, n=531), using sibling oocytes. Kitazato solutions and Cryotop® straw were used.

Assessment
Oocyte survival (1h post-warming), post-ICSI degeneration, fertilization, blastocyst development, and morphokinetics via time-lapse up to day 5.

Key Findings
- Oocyte survival rate: 98% (Group B) vs. 89% (Group A), p=0.03
- Post-ICSI degeneration: 0.8% (Group B) vs. 8.9% (Group A), p<0.001
- High-quality D5 blastocysts (4–6AA/AB): 58% (Group B) vs. 45% (Group A), p=0.04
- Cleavage timing (t2): 24.1 ± 1.2 h (Group B) vs. 25.5 ± 1.5 h (Group A), p=0.01
- D6 blastocyst rate: 5% (Group B) vs. 8.8% (Group A)
- No differences in fertilization, cleavage, euploidy, or live birth rates (p>0.05)
- Clinical pregnancy rate per single blastocyst transfer: 65.2% (Group B) vs. 54.3% (Group A)

Conclusions
These findings suggest that ultra-rapid warming protocols can optimize morphokinetic parameters, potentially improving embryo selection and IVF outcomes. Incorporating this protocol into routine practice could enhance the efficiency and success of oocyte cryopreservation cycles. This technique can eliminate the risk of micro manipulation during vitrification and warming.

Oral Communications presented in 41st ESHRE Annual Meeting at Paris (France), 2025

Transcriptomic landscape of human oocytes and trophectoderm cells following ultra-rapid or standard vitrification.

Aydin, B., Hudkova, D., Dorofeyeva, U., Pylypenko, N., Misyura, Y., Vitushynska, M., Kotliarova, O., Rozhak, N., Unsal, E., Aktuna, S., Baltaci, V., Babarikova, V., Kubaskova, T. M., Baysan, M., & Kal, N. S.

The study was conducted in collaboration with the following clinics and institutions: Ovogene Ivf clinics in Bratislava and Kosice (Slovakia); Art Clinic Ivf (Lviv, Ukraine); Ivf Georgia (Tbilisi, Georgia); Mikrogen Genetic Clinic (Ankara, Turkey); SPLN Sanatórium pre liečbu neplodnosti Ivf (Kosice, Slovakia); Istanbul Technical University, Department of Computer Engineering (Istanbul, Turkey); and Stem and Gene ART Clinic Ivf (Tirana, Albania).

View Complete Abstract

Study Design
Prospective study including 48 donor oocytes collected between January and November 2022, with sibling oocytes matched for standard or ultra-rapid vitrification and thawing, followed by ICSI and blastocyst culture to day 5/6.

Groups
Oocytes and trophectoderm cells from fresh cycles, conventional vitrification, and ultra-rapid vitrification (n=8 per cell type/protocol) were analyzed.

Assessment
Single-cell RNA sequencing (scRNA-Seq) was performed to identify differentially expressed genes (DEGs) with $|log2FC|>1$ and adjusted p-value <0.05, comparing transcriptomic profiles across groups.

Key Findings
- Conventional vitrification altered 16,016 genes in oocytes (53.18% upregulated), while ultra-rapid vitrification reduced DEGs by 97.56% (390 DEGs).
- Trophectoderm cells showed ~16,000 DEGs in both protocols; 68.16% of DEGs were downregulated in ultra-rapid versus conventional vitrification, indicating improved transcriptomic preservation with ultra-rapid vitrification.

Conclusions
This study begins to elucidate how ultra-rapid vitrification affects the transcriptomic landscape of human oocytes and embryos. Coupling the most significant DEGs with clinical data of reproductive outcomes may reveal new biomarkers for oocyte and embryo competence. This is the first transcriptomic study in the field.

Oral Communications presented in 41st ESHRE Annual Meeting at Paris (France), 2025

Ultra-Fast vs. conventional blastocyst warming: equivalent developmental outcomes following the extended in vitro culture of 221 embryos beyond the implantation stages.

Venturas, M., Ardestani, G., Zamora, M. J., Azpiroz, F., Pujol, A., Mataró, D., Rodriguez-Aranda, A., Lledó, B., Nodar, F., Miguel-Escalada, I., Sakkas, D., & Popovic, M.

The study was conducted in collaboration with the following institutions: Boston IVF – IVIRMA Global Research Alliance, Embryology (Waltham, USA); Eugin Clinic – Eugin Group, Clinical Embryology, Research and Development, and Corporate Medical divisions (Barcelona, Spain); Instituto Bernabeu, IB Biotech (Alicante, Spain); and Cegyr – Medicina y Genética Reproductiva, Eugin Group, Clinical Embryology (Buenos Aires, Argentina).

View Complete Abstract

Study Design
Prospective study including 225 vitrified blastocysts previously tested by PGT-A, categorized by chromosomal status into single trisomies (n=90), single monosomies (n=100), and euploid embryos (n=31).

Groups
Blastocysts were warmed using either conventional multi-step warming (n=118; 1-min thawing solution, 3-min dilution, 5-min washing) or Ultra-Fast one-step warming (n=107; 1-min TS, direct hold in washing solution).

Assessment
Post-warming survival and developmental progression to day 10 (D10) attachment rates were evaluated using an advanced implantation assay; stratification was performed by chromosomal status. Statistical analysis used Fisher's Exact test ($p<0.05$).

Key Findings
- Survival rates were equivalent between Ultra-Fast (98.1%) and conventional warming (98.3%, p=1.00).
- D10 attachment rates were also comparable (Ultra-Fast 57.1% vs. conventional 50.0%, p=0.35).
- Chromosomal status was a significant factor influencing attachment: euploid and trisomic blastocysts had higher attachment rates (74.4%) versus monosomic embryos (28.0%, $p<0.01$), independent of warming protocol.
- Euploid and trisomic blastocysts showed similar attachment rates between warming methods, while monosomic blastocysts had consistently lower outcomes.

Conclusions
This study underscores the value of fundamental in vitro approaches as translational research tools to validate new protocols prior to their clinical implementation. Our findings suggest that Ultra-Fast warming is an efficient alternative to conventional warming protocols, potentially streamlining IVF laboratory workflows while maintaining embryo developmental potential.

ULTRA-FAST WARM

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Oral Communications presented in 41st ESHRE Annual Meeting at Paris (France), 2025

Comparison of one-step and multi-step warming protocols: a series of 2792 blastocysts.

Aygün, T. M., Yelke, H. K., Kumtepe Colakoglu, Y., Yildiz, S., Selimoglu, S., Pirkevi Çetinkaya, C., & Kahraman, S.

The study included collaboration with the Istanbul Memorial Hospital ART and Reproductive Genetics Center, IVF Laboratory (Istanbul, Turkey).

View Complete Abstract

Study Design
Non-inferiority study with 748 embryos (alpha 5%, power 90%, control and experimental success 98%, non-inferiority margin 3%) followed by a prospective study at a single IVF center (Jan 2023-Apr 2024) with 2792 blastocysts vitrified via Kitazato protocol.

Groups
Blastocysts were warmed using either Kitazato's conventional multi-step protocol (n=1380) or Ultra-Fast warming (n=1412), including a subgroup of euploid blastocysts comparing 1M sucrose (n=877) vs Kitazato TS of 1,2M trehalose (n=853).

Assessment
Outcomes measured included clinical pregnancy rate (CPR), ongoing pregnancy rate (OPR), biochemical miscarriage rate (BMR), clinical miscarriage rate (CMR), and live birth rate (LBR). Statistical analysis was performed with Fisher's exact test.

Key Findings
- No significant differences were observed between Ultra-Fast and conventional groups in the total cohort:
  - CPR 69.1% vs. 68.9%
  - OPR 63.7% vs. 63.1%
  - BMR 7.8% vs. 7.9%
  - CMR 7.8% vs. 8.4%
  - LBR 62.1% vs. 60.9%.
- In euploid embryos, CPR was 75.8% vs. 74.0% and LBR 69.8% vs. 66.9% for Ultra-Fast and conventional groups respectively.
- The Ultra-Fast protocol significantly reduced embryo warming time, saving approximately 275 hours annually (~1 hour per workday) at the center.

Conclusions
In the complex and time-sensitive workflow of the embryology laboratory, simplifying tasks is important and may benefit to the team of embryologists in busy laboratories.

Oral Communications presented in 41st ESHRE Annual Meeting at Paris (France), 2025

Ultra-Fast vs. traditional warming: a study on human oocyte and blastocyst survival, in vitro development, and reproductive success.

Tribbioli, G., Peinado, L., Lolicato, F., Acin, L., Rovira, S., Moffa, F., Antich, M., & Novo, S.

The study involved collaboration with FERTILAB, Assisted Reproduction Unit (Barcelona, Spain) and Fertibank, Donor's Gamete Unit (Barcelona, Spain).

View Complete Abstract

Study Design
Retrospective analysis of 688 single-blastocyst cryotransfers (UFW: 317, TW: 371) and 36 ICSI cycles (UFW: 18, TW: 18; 412 MII) with donor oocytes vitrified by standard protocol in 2024.

Groups
Ultra-rapid warming (UFW) protocol (1 min thawing solution then culture medium) versus traditional warming (TW) protocol (1 min thawing solution, 3 min dilution solution, 6 min washing solutions). Kitazato solutions and Cryotop® straw were used for vitrification and warming.

Assessment
Compared survival rates, pregnancy outcomes, miscarriage rates, fertilization, ICSI degeneration, blastocyst formation, and blastocyst quality; groups were comparable in age, gamete origin, and blastocyst grade.

Key Findings
- Survival rates were similar between UFW (97.8%) and TW (98.1%; p=0.793).
- Pregnancy rates (UFW 60.9% vs. TW 61.2%; p=0.935), clinical pregnancy rates (53.0% vs. 52.3%; p=0.639), and miscarriage rates (7.1% vs. 11.3%; p=0.161) showed no statistical difference.
- Other parameters including fertilization (65.4% vs. 70.7%; p=0.383), ICSI degeneration, blastocyst formation, and high-quality blastocyst proportion were comparable.

Conclusions
Ultra-Fast Warming demonstrates comparable results to the conventional protocol and offers a promising alternative for human samples. Its ability to maintain outcomes while streamlining processes suggests potential benefits for improving laboratory efficiency and resource management in assisted reproduction practices.

ULTRA-FAST WARM

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Selected Scientific Articles: Ultra-Fast Vitrification

Costa-Borges, N., Matia-Algué, Q., Coello, A., Mestres, E., Acacio, M., Flores-Saiffe Farias, A., Castello, C., Gallardo, M., Chavez-Badiola, A., Marco-Jiménez, F., Cobo, A., & Cohen, J. (2025). **Preclinical validation of fast oocyte vitrification and warming protocols with comparable efficiencies to a standard method**. *Human Reproduction.*
https://doi.org/10.1093/humrep/deaf069

Schiewe, M. C., Reichelderfer, R., Wozniak, K., De Romana, C., Nordbak, M., Baek, K., & Chung, K. (2024). **Ultra-Fast vitrification and rapid elution of human oocytes: Part I. Germinal vesicle model validation**. *Reproductive Biomedicine Online, 49(6), 104691.*
https://doi.org/10.1016/j.rbmo.2024.104691

Wozniak, K., Reichelderfer, R., Ghaemi, S., Hupp, D., Fuzesi, P., Ringler, G., Marrs, R. P., & Schiewe, M. C. (2024). **Ultra-Fast vitrification and rapid elution of human oocytes: Part II. Verification of blastocyst development from mature oocytes.** *Reproductive Biomedicine Online, 49(6), 104690.*
https://doi.org/10.1016/j.rbmo.2024.104690

Aydin, B., Hudkova, D., Maggiotto, G., Kal, N. S., Osmanllari, U., Unsal, E., Baltaci, V., Aktuna, S., Yonar, D., Dorofeyeva, U., Kotliarova, E., Vitushynska, M., Misyura, Y., Yetim, I., & Babarikova, V. (2024). **Human oocyte survival, early embryo development, metabolic fingerprinting, and pregnancy outcomes following ultra-rapid or standard vitrification and thawing.** *Human Reproduction, 39(Suppl. 1), deae108.351.*
https://doi.org/10.1093/humrep/deae108.351

Costa-Borges, N., Coello, A., Cohen, J., Gallardo, M., Flores-Saiffe Farias, A., Mestres, E., Acacio, M., Matia-Algué, Q., Castello, C., Calderón, G., Chavez-Badiola, A., & Cobo, A. (2024). F**ast vitrification and warming protocols demonstrate similar efficiencies to a standard method and a substantial reduction in execution times.** *Human Reproduction, 39(Suppl. 1), deae108.352.*
https://doi.org/10.1093/humrep/deae108.352

Cascales, L., Herrero, L., Aparicio, M., Boyano, B., Garcia, A., Ten, J., Bernabeu, A., & Bernabeu, R. (2024). **Evaluation of Ultra-Fast oocyte vitrification and warming method: Preliminary results.** *14th Biennial Alpha Conference, Lisbon, Portugal.*
https://doi.org/10.1016/j.rbmo.2024.104061

Gudkova, D., Aydin, B., Dorofeyeva, U., Karimova, H., & Kal, N. S. (2024). **Clinical and metabolic effects of ultra-rapid vitrification and thawing on oocyte and embryo viability.** *80th Annual Meeting of the American Society for Reproductive Medicine (ASRM), Denver, CO, United States.*
https://doi.org/10.1016/j.fertnstert.2024.07.640

Inoue, F., Acacio, M., Mestres, E., Matia-Algué, Q., Gago, S., Castelló, C., Ogawa, M., & Costa-Borges, N. (2025). **A novel one-step oocyte vitrification protocol shows promise as a simpler and faster alternative to standard methods.** *41st Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), Paris, France.*

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Selected Scientific Articles: Ultra-Fast Warming

Ezoe, K., Miki, T., Fujiwara, N., & Kato, K. (2025). **Influence of the shortened warming protocol on human blastocyst viability: An in-vitro experimental study.** *Reproductive BioMedicine Online, 1, 104454.*
https://doi.org/10.1016/j.rbmo.2024.104454

Manns, J. N., Katz, S., Whelan, J., Patrick, J. L., III, Holt, T., Merline, A. M., & Taylor, T. H. (2021). **Validation of a new Ultra-Fast blastocyst warming technique reduces warming times to 1 minute and yields similar survival and re-expansion compared to blastocysts warmed using a standard method.** *77th Annual Meeting of the American Society for Reproductive Medicine (ASRM), Baltimore, United States.*
https://doi.org/10.1016/j.fertnstert.2021.07.456

Bronet, F., Coello, A., Murria, L., Garijo, Y., Martínez Méndez, E., Garcia-Velasco, J. A., & Cobo, A. (2024. **Rapid warming protocol of human oocytes: A randomized controlled trial.** *80th Annual Meeting of the American Society for Reproductive Medicine (ASRM), Denver, United States.*
https://doi.org/10.1016/j.fertnstert.2024.07.647

Zamora, M. J., Gayete-Mor, B., Regincós, M., Quintana-Vehí, A., Pujol, A., Mataró, D., Rodríguez-Aranda, A., Miguel-Escalada, I., & Popovic, M. (2025). **One-step Ultra-Fast warming of vitrified human oocytes: Comparable clinical outcomes and improved blastocyst rates in a sibling oocyte study.** *41st Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), Paris, France.*

Regincós, M., Zamora, M. J., Correa, N., Quintana-Vehí, A., Pujol, A., Mataró, D., Rodríguez-Aranda, A., Miguel-Escalada, I., & Popovic, M. (2025). **Comparative analysis of one-step Ultra-Fast versus multi-step conventional warming protocols: A retrospective study of 2,548 single frozen embryo transfers.** *41st Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), Paris, France.*

Yaacobi-Artzi, S., Yonish, M., & Shavit, T. (2025). **Ultra-Fast warming protocol for vitrified blastocysts demonstrates similar survival, re-expansion, and pregnancy rates compared to the standard warming protocol.** *41st Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), Paris, France.*

ULTRA-FAST WARM

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