RAPIDVIT & RAPIDWARM OOCYTE

Specialised media for oocyte vitrification.

Vitrolife

SPECIAL MEDIA FOR A UNIQUE CELL

Cryopreservation of oocytes requires care. Some preservation techniques cause premature oocyte activation and zona hardening that can hinder the success of IVF. However, with the correct media and technique, oocyte preservation has become a reliable routine.

Large, fragile and primed for action

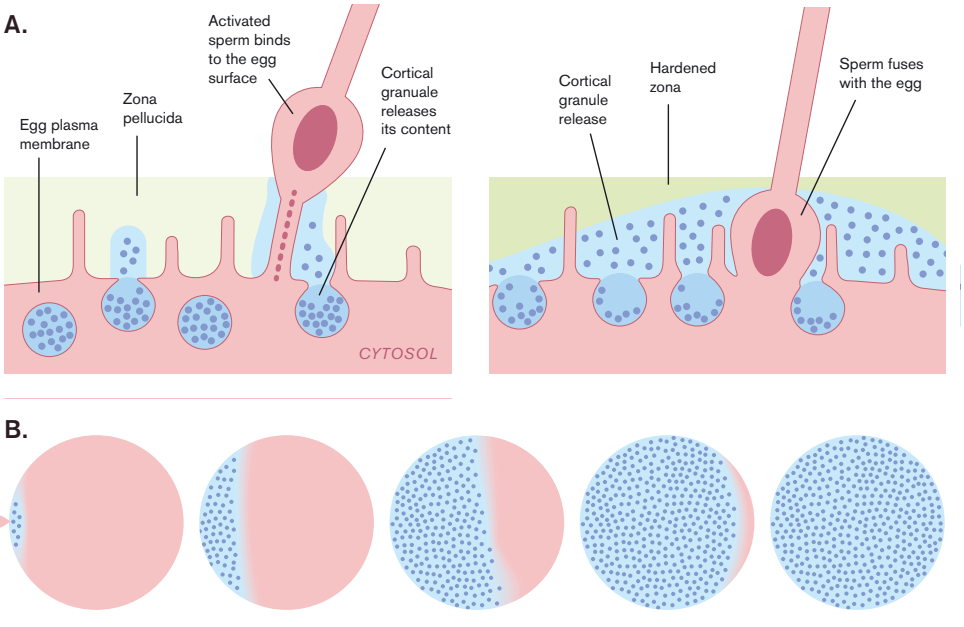
Oocytes are the largest cell a human body can produce. They are packed with delicate organelles and subcellular structures such as the meiotic spindle, actin filaments and cortical granules. Due to their size (~ 125 µm) and spherical shape they have a low surface-to-volume ratio.

Unlike multicellular embryos, oocytes have presented special challenges to cryopreservation. As single, water-filled cells with different membrane permeability they offer an uncompromising all-or-nothing scenario during preservation. The zona and spindle are sensitive structures and care must be taken in selecting the right methodology and cryoprotectants to prevent physical and chemical injury.

The calcium trigger

Under in vivo conditions oocytes are activated by a series of events that are initiated by a fertilising sperm (Fig 1A). The sperm causes a precise pattern of intracellular free calcium oscillations that play a crucial role in oocyte activation (Fig 1B). The initial calcium increase lasts about 5 minutes and prompts the cortical granules to release activating proteins and enzymes. This results in zona hardening as part of the blocking mechanism against polyspermy.

Fig 1. Fertilisation triggers the calcium wave



Oocyte activation and calcium
An increase in intracellular calcium is initiated by sperm–egg fusion. Zona hardening is brought about by fusion of cortical granules to the plasma membrane and the release of their contents into the zona pellucida layers.

The calcium wave
Upon fusion, the sperm triggers a series of calcium oscillations.

Certain cryopreservatives are effective in triggering calcium release[1,2]. This can induce zona hardening and even artificially activate oocytes prior to fertilisation.

RapidVit Oocyte keeps calcium under control

RapidVit Oocyte was developed after extensive research on the effects of cryoprotectants on oocytes and their intracellular calcium content. Studies show that careful control of intra- and extracellular calcium has a significant impact on the success of cryopreservation and oocyte viability[1, 2].

RapidVit Oocyte is a calcium-free medium based on the G-Series media and uses ethylene glycol (EG) and propanediol (PrOH) as cryoprotectants. The effect of the cryoprotectant is reduced when calcium is omitted, (Fig 2D), especially when compared to other common cryoprotectants (Fig 2 A, B and C)[1, 2]. RapidVit Oocyte is designed to minimise the calcium release preventing the negative effects of artificial activation.

Work with convenience, speed and stability

Convenience The media are ready-to-use which saves time and give consistent results. Getting started is simple as no time is wasted on mixing solutions.

Speed By working at a constant 37°C, processes are speeded-up to allow vitrification of oocytes in less than 10 minutes. Moreover oocytes can be prepared and transferred in a standardised way and without fuss.

Stability Each vitrification step is conducted in large volumes rather than drops, so the temperature and media remain stable due to negligible evaporation.

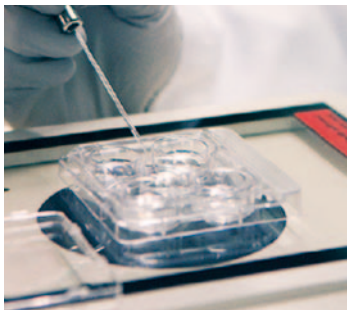
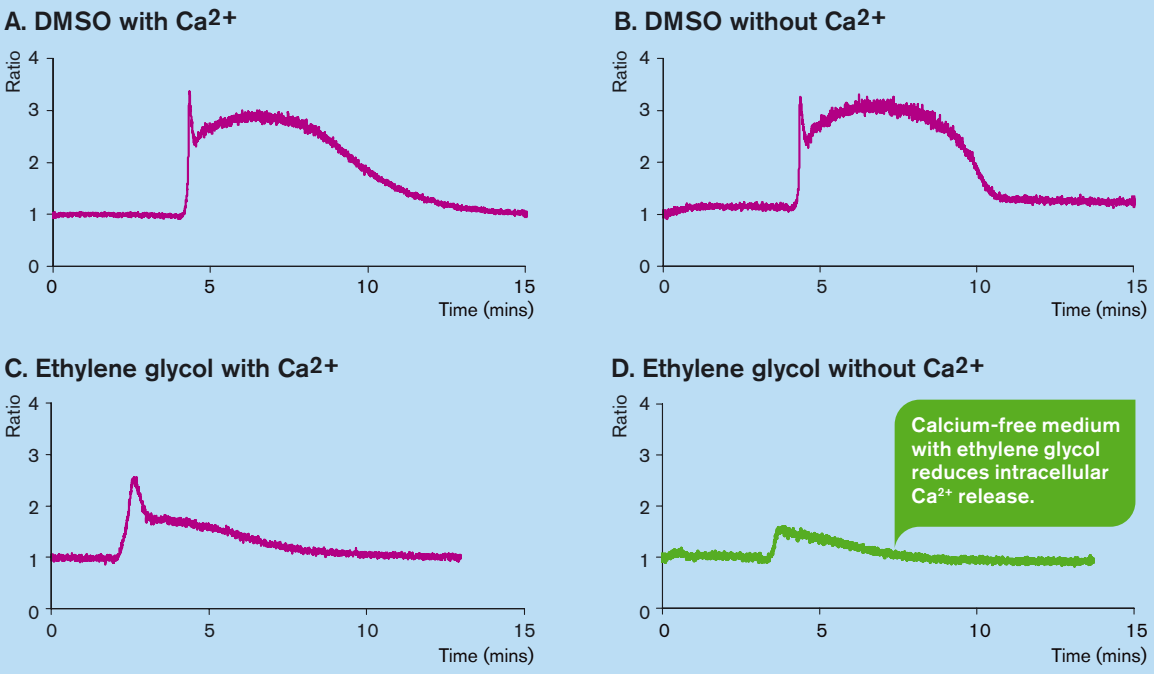


Fig 2. Calcium-free, ethylene glycol based media minimise the release of free intracellular Ca^{2+} to reduce the risk of artificial activation. This has a positive effect on oocyte viability.



CLOSING THE OPEN ISSUE

Rapid-i Vitrification System works as well as open systems at all developmental stages.

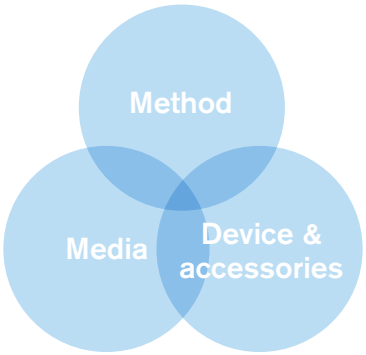
Aseptic and effective

Numerous publications and trials have dispelled concerns about the necessity of using open systems to achieve successful vitrification. As a closed system, Rapid-i Vitrification System reduces the risk of contamination and physical harm from long-term contact with liquid nitrogen during cooling and storage.

The Rapid-i device enables instant warming of oocytes through direct contact with RapidWarm Oocyte at 37°C. This is an advantage since the rate of warming after vitrification has been shown to have a positive effect on survival rates[3].

A caring system that delivers

Many independent studies that compare open vitrification systems with Rapid-i indicate that Rapid-i provides successful vitrification and safe storage for embryos at all developmental stages.



Results from post-warming show that: survival rates can be as high as ≥95%, good blastocyst development, implantation rates ranged from 31-52%, and clinical pregnancy rates from 52-57%. [4, 5, 6, 7, 8, 9, 10, 11, 12, 13]

Two comparative studies clearly show that Rapid-i can perform equally well as an open vitrification system. In the first study[14], no significant differences were found between CryoTop and Rapid-i when in-vitro cell damage (Fig 3), and developmental outcome after transfer to patients (Fig 4) were compared.[11]

Fig 3. Cell membrane damage – as safe

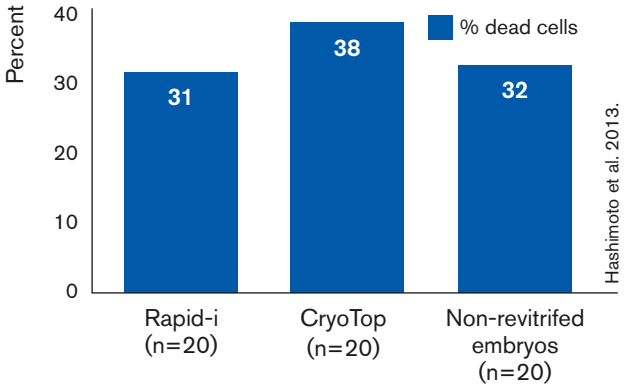
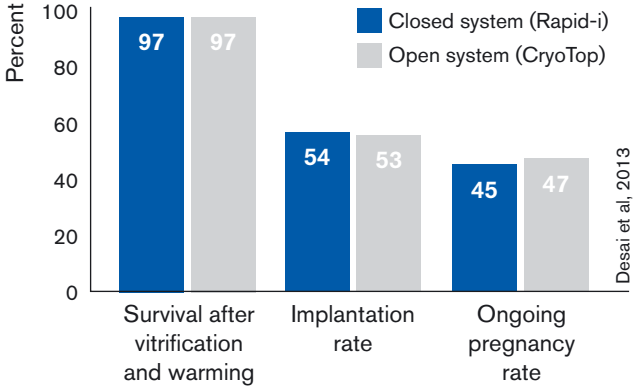


Fig 4. Implantation and pregnancy rates – as successful



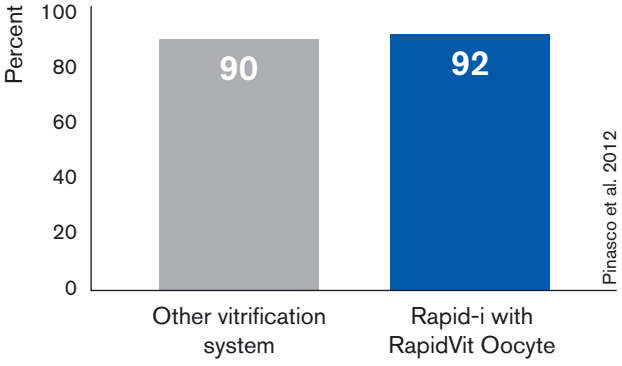
EXCELLENT VIABILITY

The Rapid-i Vitrification System together with RapidVit/Warm media provides excellent viability with safe and aseptic workflow.

High survival rates and low risks

Rapid-i Vitrification System avoids the risks associated with liquid nitrogen that are found with open systems. Pinasco et al[15] conducted a prospective randomised trial to compare oocyte survival rates with open and closed devices. In total 193 sibling oocytes were randomised to vitrification/warming in one of two groups (Fig 5). The authors conclude Rapid-i with RapidVit and RapidWarm Oocyte media gave survival rates similar to another vitrification system (open).

Fig 5. Oocyte survival rates after vitrification



As good as fresh oocytes

Machač et al.[16] performed a study comparing outcomes from vitrified and fresh donor oocytes. Embryos were transferred on day 5. The results showed that post-warming survival was equivalent to results in the published literature (Fig 6).

The clinical parameters; fertilisation rate, implantation rate and clinical pregnancy rate gave comparable results in both groups (Fig 7). The authors conclude that vitrified oocytes using the Rapid-i Vitrification System and RapidVit Oocyte are as viable as those derived from fresh oocytes.

Fig 6. Survival after vitrification with RapidVit media and Rapid-i Vitrification System is equivalent to previously published results

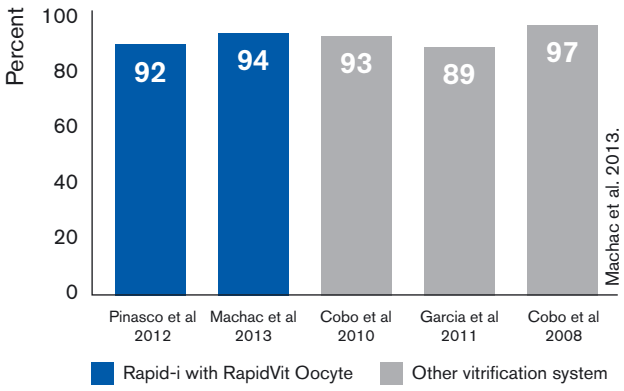
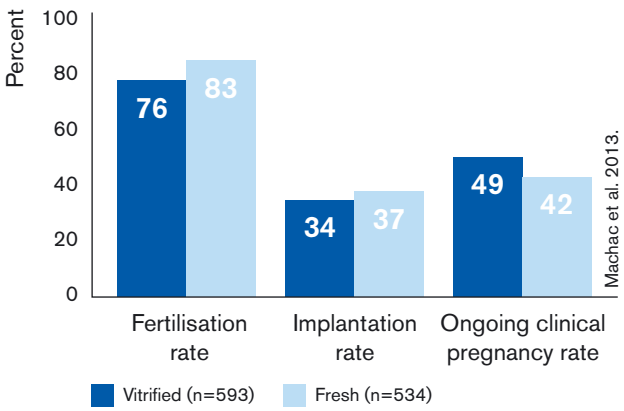


Fig 7. Vitrified and fresh oocytes give comparable outcomes



SYNCHRONISED FOR SUCCESS

Rapid Vit/Warm Oocyte media together with the Rapid-i Vitrification System provides consistent results with less stress.

Media optimised for oocytes

RapidVit/Warm Oocyte media are designed to optimise oocyte survival and viability after vitrification and warming. The media support minimal release of calcium to prevent premature oocyte activation. Rapid Vit/Warm Oocyte media also support metabolism with amino acids, carbohydrates, and human serum albumin (HSA). Hyaluronic acid increases viscosity and helps yield higher survival rates and improved in vitro embryo development. The media are buffered with MOPS and are ready to use after warming to 37°C.

Reliability at every step

Rapid-i Vitrification System combines effective vitrification and instant warming without direct exposure to liquid nitrogen. This minimises the risks of contamination and harm from the coolant. Furthermore, the Rapid-i workflow allows you to vitrify, seal and store without stress. Oocytes are easily placed in the Rapid-i device for vitrification and are handled in an unbroken cold chain until they are needed again. SmartBox helps you stay organised and prevents unintentional warming by holding storage straws safely in place.

Product	Description	Size	REF
Rapid-i™ Kit	Vitrification device *	20-pack	14406
Rapid-i™ Cutter	Cutter	1-pack	14413
Rapid-i™ Forceps	Forceps	1-pack	14410
Rapid-i™ Sealer	PS-202, 120V	1-pack	14414
Rapid-i™ Sealer	PS-202, 230V	1-pack	14415
Rapid-i™ Goblet	Plastic tube to hold Rapid-i™ Kit	20-pack	14416
Rapid-i™ CryoCane	For storage of Rapid-i™ Kit in cryotank, holds 1 goblet	20-pack	14417
SmartBox™	Box for liquid nitrogen, used with Rapid-i™ tools	1-pack	14408
RapidVit Blast™	For vitrification of blastocysts	3 x 10 mL	10119
RapidWarm Blast™	For warming of vitrified blastocysts	3 x 10 mL	10120
RapidVit Cleave™	For vitrification of cleavage stage embryos	3 x 10 mL	10117
RapidWarm Cleave™	For warming of vitrified cleavage stage embryos	4 x 10 mL	10118
RapidVit Oocyte™	For vitrification of oocytes	3 x 10 mL	10121
RapidWarm Oocyte™	For warming of vitrified oocytes	4 x 10 mL	10122

* In the US - for 4-8 cell stage embryos only

Vitrolife
TOGETHER. ALL THE WAY™

Vitrolife · Box 9080 · SE-400 92 Göteborg · Sweden · Tel +46 31 721 80 00 · Fax +46 31 721 80 90 · fertility@vitrolife.com · www.vitrolife.com

REFERENCES **1**. Larman MG, et al. Calcium-free vitrification reduces cryoprotectant-induced zona pellucida hardening and increases fertilization rates in mouse oocytes. Reproduction. 2006 Jan;131(1):53-61. **2**. Larman MG, et al. 1,2-propanediol and the type of cryopreservation procedure adversely affect mouse oocyte physiology. Hum Reprod. 2007 Jan;22(1):250-9. Epub 2006 Aug 12. **3**. Seki S and Mazur P. The dominance of warming rate over cooling rate in the survival of mouse oocytes subjected to a vitrification procedure. Cryobiology. 59: 75-82, 2009. **4**. Seida K, et al. Evaluation of the ultra-rapid method for vitrification using Rapid-i™. Presented at the Japan Society for Reproductive Medicine, Kanto region, 2011. **5**. Hoiruchi R, et al. Development of human embryos after freezing-thawing by Rapid-i™ method. Presented at the Japanese Society of Reproduction Engineering, 2012. **6**. Mizuno S, et al. Comparison of open and closed devices for vitrification of human embryos. Presented at the 4th Congress of the Asia pacific Initiative on Reproduction (ASPIRE), 2012. **7**. Ohsumi K, et al. Effectiveness of the closed vitrification device in frozen-thawed embryo transfer. Presented at the 4th Congress of the Asia pacific Initiative on Reproduction (ASPIRE), 2012a. **8**. Amo A, et al. A closed vitrification system enables a safe and an aseptic vitrification without impairing the developmental competence of human embryos. Presented at the ASRM meeting, 2012. Fertility and Sterility, 98, 3, Supplement, S124, P-40. **9**. Ohsumi K, et al. The search for the next-generation of cryo preservation system: Examination of the closed vitrification device. Presented at the Japanese Society of mammalian Ova Research, 2012b. **10.** Desai N, et al. The new Rapid-i™ carrier is an effective closed system for human embryo vitrification of both the blastocyst and cleavage stage. Presented at the ESHRE meeting, 2012. Human Reproduction, 27, Supplement 2, ii59-60, O-154, 2012a. **11.** Desai et al. The new Rapid-i carrier is an effective system for human embryo vitrification at both the blastocyst and cleavage stage. Reproductive Biology and Endocrinology 2013 11:41. **12.** Egashira A, et al. Evaluation of cryopreservation of a small number of sperm in a non-liquid nitrogen contact system. Presented at the Japan Society of Fertilization and Implantation, 2011. **13.** Otsubo H, et al. Croypreservation of a small number of human spermatozoa in a closed system by using Rapid-i™. Presented at the ESHRE meeting, 2011. Human Reproduction, 27, Supplement 1, i181-182, P-150, 2011. **14.** Hashimoto S et al. A closed system supports the developmental competence of human embryos after vitrification. J Assist Reprod Genet. 2013 Mar;30(3):371-6. doi: 10.1007/s10815-012-9928-2. Epub 2013 Jan 12. **15.** M. Pinasco et al. Oocyte Vitrification Freeze/Thaw Survival Rates Using an Open Versus a Closed System. Fertility and Sterility Vol 97 No 3 Supplement S-18, P-27, 2012 **16.** Machač et al. A novel method for human oocyte vitrification with a closed device using super-cooled air. Fertility and Sterility Vol 100 No 3 Supplement S108, O-353, 2013.