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Summary of abstracts regarding single-step rapid warming

A selection of presented research

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48TH BSRM SCIENTIFIC MEETING 2022

Poster P07 Multicentre pre-clinical validation of warming procedures for human blastocysts involving a short exposure to a single sucrose solution shows promising survival, re-expansion and continued development.

Gunst J., Ahlstrom A., AZ Sint-Jan Brugge-Oostende av, Laboratory Medicine, Bruges, Belgium; Livio, GeneraLife IVF, Gothenburg, IVF laboratory, Gothenburg, Sweden.

Objective:
For vitrification and warming of blastocysts, commercial kits with differences in types and concentrations of cryoprotectants and warming temperature are available. Warming procedures usually involve consecutive steps of exposure to solutions with decreasing concentrations of non-penetrating cryoprotectants. Comparisons of sucrose concentrations between kits indicate that simpler warming procedure may be equally effective. Moving towards simpler protocols can support further optimisation of laboratory procedures.

Design and methods:
In two IVF laboratories, human blastocysts consented by patients for validation purposes were used for warming in a single step, using different warming solutions and exposure times. Blastocyst collapse was performed before vitrification. Depending on the kit used clinically, embryos were either warmed in the corresponding warming kit and re-vitrified or warmed directly according to the modified procedure. Vitrified blastocysts were plunged into a warming solution at 37°C, containing low or high sucrose (RapidWarm Omni, Warm 3 or Warm 1 respectively) and stayed in the respective solutions for 2 or 1 minutes. Blastocysts were then transferred into isotonic medium, cultured following several rinsing steps and assessed for morphological survival. Re-expansion and further development were assessed up to 24 h post warming. When available, warmed embryos were cultured in a time-lapse incubator, allowing objective assessment of time to re-expansion and time to hatching. Control embryos were treated following standard warming times.

Results:
Results on number of embryos, survival, re-expansion (< 5 h) and development after 24 h are summarised in the table. Results were similar for high and low sucrose and not different compared to the standard warming procedure.

	Low sucrose	High sucrose
Number of warmed/ recovered (A)	52/50	50/50
Survived (% of A)	50 (100)	49 (98)
Re-expanded (% of A)	49 (98)	47 (94)
24 h hatching/hatched (% of A)	37 (74)	38 (78)

Culture after warming in low sucrose medium shows a tendency to faster re-expansion (1.64 ± 0.61 h versus 2.2 ± 1.41 h) and time to hatching (5.1 ± 0.8 h versus 7.82 ± 13.51 h).

Conclusions:
Results confirm that single-step warming of blastocysts at 37°C yields survival and development rates similar to multistep warming procedures. A trend towards earlier resumption of functionality is observed after warming in low-sucrose medium. Pre-clinical data indicate no negative effect of a faster warming procedure, but this has to be confirmed clinically. A short warming procedure has the potential to support further laboratory workflow optimisation.

65th AAB Conference 2023

Rapid warming of human blastocysts: Is 1M sucrose the only choice?

Liebermann, J.,[1] Wagner Y.,[1] Pantos M.,[1] Susralski A.,[1] Brohammer R.,[1] Joris H.[2]

[1] Fertility Centers of Illinois, Chicago, Illinois
[2] Vitrolife Sweden AB, Gothenburg, Sweden

Objective:
Over the last six months a new rapid warming protocol for vitrified human blastocysts has found to be equivalent to the traditional warming protocols, which uses decreasing sucrose concentrations over several minutes. The rapid warming protocol exposes blastocysts to 1M sucrose for 1 minute after which the cells are ready for transfer. The use of sucrose during warming protocols is to control the influx of water into the cell compartments. However, the abstract presented here investigated if lower sucrose concentrations are also capable of controlling the water influx. Lower sucrose concentration would allow a faster return of water and therefore physiological conditions.

Design:
Retrospective study using human blastocysts donated by patients with signed disposition for research.

Material and methods:
Using Vitrolife warming solution of 0.5M (Protocol 1=P1) and 0.25M (Protocol 2=P2) sucrose, blastocysts were exposed for 1 minute to either concentration. Immediate post-warming as well as 24hrs post warming survival and hatching/hatched rate were recorded. The Chi2 was used to determine statistical significance between the two groups, with $P<0.05$ considered significant.

Results:
A total of 202 blastocysts were rapid warmed. 102 blastocysts for 1 min in P1, whereas 100 blastocysts were warmed for 1 minute in P2. There was no significant difference in immediate post-warming survival between P1 vs P2 (97% vs 97%; p=0.99). However, there was a significant difference in the 24hrs post-warming hatching/hatched rate between P1 vs. P2 (77.3% vs 100%; p=0.0006).

Conclusions:
This study provides evidence that 1 minute exposure to 0.5 or 0.25M sucrose can support survival rates equivalent to 1M sucrose. However, the data suggests that 0.25M sucrose could allow faster recovery as exhibited by the significantly higher hatching/hatched rate.

Disclosures:
Nothing to disclose

Funding:
none

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Rapid warming of non-biopsied vs biopsied blastocysts: Is there a need for assisted hatching?

Liebermann J.,[1] Wagner Y.,[1] Pantos M.,[1] Susralski A.,[1] Brohammer R.,[1] Joris H.[2]

[1] Fertility Centers of Illinois, Chicago, Illinois
[2] Vitrolife Sweden AB, Gothenburg, Sweden

Objective:
Recently ASRM released guidelines in conjunction with assisted hatching, a procedure where the zona pellucida (ZP) of a blastocyst will be breached by employing a laser system. ASRM suggested to be more conservative using assisted hatching. However, recent publications have shown that especially cryopreserved embryos develop a change in their ZP structure involving the three ZP proteins also known as “Zona Hardening”. This phenomenon makes it harder for the embryo to hatch out of the zona on their own. However, hatching out of the ZP is a requirement for an embryo to implant successfully. One way to look at it is comparing the ability of non-biopsied vs biopsied embryos and their ability to hatch on their own without employing a laser system post-warming.

Design:
Retrospective study using human blastocysts donated by patients with signed disposition for research.

Material and methods:
Donated human blastocysts were divided in group A (n=49; biopsied blastocysts) and group B (n=53; non-biopsied blastocysts) and were then rapid warmed for 1 minute using Vitrolife's 0.5M warming solution. 24hrs post warming survival and hatching rate as well as the rate of completely hatched blastocysts were recorded. The Chi2 was used to determine statistical significance between the two groups, with P<0.05 considered significant.

Results:
After 24 hrs post-rapid warming, survival in group A was 95.9% (47) vs group B were 94.3% (50), retrospectively (P=.0.99). In addition, blastocysts in group A hatched at a significantly higher rate (91.4% (43) than blastocysts in group B (64.0% (32); (p=0.001). Furthermore, looking at the rate of embryos completely hatched out of the zona, 68.1% (32) in group A vs 22.0% (11) of embryos in group B hatched completely (P<0.0001).

Conclusion:
This study shows that human blastocysts regardless of being biopsied or not, survive at a high rate after a rapid warming in 0.5M sucrose. While embryos in both groups maintain their ability to start hatching and completely hatch out of the zona, non-biopsied embryos initiated hatching or hatched completely at a significant lower rate compared to their counterpart, which suggests the consideration of assisted hatching in non-biopsied blastocysts to support their ability of a successful implantation.

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Ultra-rapid warm: A preclinical comparative analysis of the effects of different sucrose concentrations on blastocyst survival, re-expansion and development post-warm

Emeny-Smith K.,[1] Proctor J.G.,[1] Larman M.G.,[2] Kirkpatrick S.[3]

[1] Conceptions Reproductive Associates of Colorado, [2] Vitrolife AB Sweden, [3] Vitrolife USA
[1] Littleton, Colorado, [2] Gothenburg, Sweden and [3] Englewood, Colorado

Objective:
To assess the viability of time-efficient, ultra-rapid embryo warming by studying blastocyst survival, re-expansion, and development following a 1-minute exposure to either 1.0, 0.5, or 0.25M sucrose solutions.

Design:
An exploratory study designed to review time-efficient blastocyst warming and identify the optimal sucrose concentration to inform further clinical study.

Materials and methods:
153 human blastocysts previously vitrified using RapidVit Blast media (Vitrolife) and Rapid-i (Vitrolife) were used for this study. All blastocysts were consented by patients for research use by the laboratory and were of A or B (Gardner scale) quality at the time of cryopreservation. 51 blastocysts were assigned to each sucrose solution (1.0, 0.5 and 0.25M) from RapidWarm Oocyte kit (Warm 1, 2 and 3, respectively; Vitrolife). Vitrified blastocysts were plunged into a 37°C warming solution containing a precise concentration of sucrose. Blastocysts remained in the respective solution for 1 minute before being transferred to a 20% protein blastocyst culture medium. Blastocysts were thoroughly rinsed through multiple drops of medium before being cultured in groups and assessed for survival, re-expansion (<3 h post-warm) and blastocyst development (24h post-warm).

Results:
Cryopreservation survival was consistent with accepted lab standard among all sucrose concentrations. There were no statistical differences, but the trend was that 1.0M sucrose resulted in increased re-expansion and the best 24 hour post-warm development when compared to 0.5 sucrose and 0.25M sucrose.

	1.0M Sucrose (Warm 1)	0.5M Sucrose (Warm 2)	0.25M Sucrose (Warm 3)
# of Embryos	51	51	51
# Survived	51 (100%)	50 (98%)	49 (96%)
# Re-expanded (<3h post-warm)	49 (96%)	46 (90%)	46 (90%)
24h Post-warm development	49 (96%)	46 (90%)	44 (86%)

Conclusions:
Increased workloads and additional workflows of the modern IVF laboratory demand improved efficiency. This study tested an ultra-rapid, single-step blastocyst

warming technique. All 3 sucrose concentrations supported high survival and subsequent development. Of the three solutions tested, 1.0M sucrose (Warm 1) resulted in the fastest re-expansion time and the most promising post-warm development, although this observation requires further exploration and clinical outcome data. These results suggest that ultra-rapid warming in a high sucrose concentration warming solution shows an encouraging outcome to aid in improving lab workflow optimisation and efficiency while maintaining or exceeding current laboratory standards for embryo survival, re-expansion, and post-warm development.

Disclosures:
Nothing to disclose

Funding:
None

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Evaluation of a rapid warming protocol for human vitrified blastocysts

Van Landuyt L., Van Asbroeck J., Wouters K., De Munck N.

Universitair Ziekenhuis Brussel, Brussels IVF, Laarbeeklaan 101, 1090 Brussels, Belgium

Aim of the study:
To investigate if a rapid 2 min warming protocol for vitrified blastocysts can result in similar survival rates and post warming development as obtained by our standard warming procedure.

Study design:
Single center prospective pilot study on 120 vitrified blastocysts released for scientific research. Both day 5 and day 6 blastocysts were included originating from standard IVF/ICSI cycles and cycles where PGT was applied. The standard warming protocol was compared with 2 rapid warming protocols with low sucrose. Patients with at least 3 blastocysts vitrified for research were included to be able to compare the 3 protocols within the same patient.

Materials and methods:
Blastocysts were vitrified using CBS-VIT-HS closed vitrification straws and Irvine Scientific freeze media (Vit kit for non-PGT blastocysts and VitNX kit for PGT blastocysts). For the standard warming protocol, Irvine Scientific warming media were used, described by Van Landuyt et al. 2015[1]. This consists of a 12 min protocol starting with incubation in a 250 µl droplet of 1 M sucrose at 37°C , followed by incubation at room temperature in 0,5M sucrose and several washing droplets without sucrose. The rapid protocol involves a 2 min incubation at 37°C in 250 µl or 500 µl 0,25 M sucrose (RapidWarm Omni Warm 3, Vitrolife) followed by 3 washing steps in blastocyst culture medium (Origio). After warming, blastocysts were incubated in Embryoscope (Vitrolife) for 24 hours.

Outcome parameters:
Total survival rate (≥50% intact) and fully intact survival rate (100% intact) were assessed immediately after warming. Re-expansion rate was assessed after 2h-4h and further development (hatching/hatched rate)

Results:

	IVF/ICSI blastocysts (non-PGT)				PGT blastocysts			
	Standard	Rapid*	Rapid 250 µl	Rapid 500 µl	Standard	Rapid*	Rapid 250 µl	Rapid 500 µl
N warmed	20	40	20	20	20	40	20	20
N survived (%)	20 (100)	40 (100)	20 (100)	20 (100)	20 (100)	40 (100)	20 (100)	20 (100)
N fully intact (%)	20 (100)	32 (80)	16 (80)	16 (80)	16 (80)	31 (77.5)	15 (75)	16 (80)
N re-expanded 2h (%)	15 (75)	27 (67.5)	13 (65)	14 (70)	14 (70)	26 (65)	14 (70)	12 (60)
N re-expanded 4h (%)	20 (100)	38 (95)	19 (95)	19 (95)	17 (85)	28 (70)	15 (75)	13 (65)
N no re-expansion (%)	0 (0)	2 (5)	1 (5)	1 (5)	2 (10)	8 (20)	2 (10)	6 (30)
N hatching 4h (%)	16 (80)	23 (57.5)	12 (60)	11 (55)	16 (80)	28 (70)	15 (75)	13 (65)
N hatching/hatched 24h (%)	19 (95)	38 (95)	19 (95)	19 (95)	19 (95)	32 (80)	16 (80)	16 (80)

* sum of Rapid 250 µl and Rapid 500 µl

was assessed up to 24 hours after warming. Also the percentage of blastocysts that never showed re-expansion was assessed.

Conclusion:
"This pilot study shows that similar survival rates and post warming development can be obtained by shortening the warming protocol to 2 min in low sucrose. Further clinical validation is required to evaluate pregnancy and implantation rates.

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Validation of single-step warming for human blastocysts shows successful results are independent of the sucrose concentration used

Abu Ali K.[1], Ahlström A.[2]

[1] Livio- GeneraLife IVF- American Hospital- Dubai, IVF department, Dubai, United Arab Emirates
[2] Livio- GeneraLife IVF, IVF department, Gothenburg, Sweden

Study question:
Can warming of blastocysts in a single step be successfully performed using different sucrose concentrations?

Summary answer:
Blastocyst warming in a single step using sucrose concentrations between 0.25 M and 1.0 M shows similar rates of survival, re-expansion and development in vitro.

What is known already:
Since the introduction of human blastocyst vitrification, excellent survival rates have been reported. Warming procedures usually involve three to five steps with exposure to solutions with decreasing concentrations of non-penetrating cryoprotectants. Successful blastocyst warming procedures are described using high as well as low starting levels of sucrose. Recent data show that a single step warming procedure can be equally effective in terms of survival and pregnancy rates. Moving towards simpler procedures can support further optimization of laboratory procedures.

Study design, size, duration:
Blastocysts donated and consented by patients were used to evaluate a shorter warming procedure. Warming was performed in a single step using different sucrose concentrations. After warming, embryos were assessed for morphological survival and cultured in a time-lapse incubator to monitor re-expansion and development in vitro for 24 h. Three series of tests were performed. A control group using standard warming procedure was included in the first test.

Participants/materials, setting, methods:
For warming, carriers containing a single blastocyst were plunged into a warming solution at 37 °C, containing 0.25, 0.5 or 1.0 M sucrose and stayed in the solution for 1 or 2 minutes. Three series of experiments were performed, testing different sucrose concentrations or volume of warming solution. Warmed blastocysts were cultured following several rinsing steps and assessed for morphological survival. Re-expansion after 2 h, embryo characteristics and development were monitored until 24 h post warming.

Results:

	Test 1		Test 2		Test 3		
Exposure time	Control: 2'+3'+5'	2'	1'	1'	1'	2'	1'
Sucrose level (M)	0.25, 0.125, 0	0.25	1	1	0.5	0.25	0.25
Warming volume (mL)	1	1	1	0.2	1	1	1
Warmed	10	20	20	20	10	10	10
Recovered	10	19	19	20	10	10	10
Transferable	9	19	19	20	10	10	10
Re-expanded in 2 h	7	15	15	17	7	9	7
24 h survival	9	19	19	19	10	9	10

Main results and the role of chance:
Results of the different tests are summarized in the table, including information on sucrose levels, exposure times, volume of warming medium, numbers of blastocysts warmed, recovered, meeting criteria for embryo transfer, full re-expansion after 2 hours, viable after 24 h culture.

No differences were observed between the different groups. Overall, 98 % of warmed blastocysts would be considered transferable after single step warming and 96 % were viable after 24 h culture. Results confirm blastocysts can be warmed at 37 °C in a single step using 0.25 M, 0.5 M or 1 M sucrose with no effect on survival or development in vitro.

Limitations, reasons for caution:
This is a preclinical validation on use of a reduced warming time for vitrified blastocysts. Further evaluation and clinical validation of the findings is required to confirm safety of a simplified warming procedure. Working temperature rather than warming medium volume may be critical when aiming for shorter warming procedures.

Wider implications of the findings:
Reducing the warming time and number of handling steps minimizes exposure of blastocysts to a suboptimal environment and operator or handling related stresses. It also allows further optimization of laboratory protocols and workflow. When confirmed clinically, these findings encourage investigating similar changes for other reproductive cells.

Trial registration number:
not applicable

Reflections on single-step rapid warming

"As time-efficiency is a critical parameter in overbusy IVF labs, the recent onset of ultra-fast warming procedure was definitely a great opportunity that we decided to explore immediately. This was obviously extremely simple to implement, and it rapidly confirmed its expected capacity to reduce time and to ensure smooth workflow, while providing perfectly stable and maintained clinical outcomes, as confirmed after more than 700 cycles."

Prof Thomas Freour, Chef de service – Biologie et Médecine de la Reproduction, gynécologie médicale, Responsable du centre AMP, CHU de Nantes

"The implementation of rapid warming in our blastocyst cryopreservation, and warming program is a game changer in many ways: Foremost a time saver; thawing 1 minutes and then ready for transfer is in a big program like ours, a huge improvement in our daily schedule. On top, allowing blastocyst to reach their physiologically intracellular milieu after warming faster "makes" them healthier too, which is reflected in better outcomes such as higher ongoing pregnancy rates, and lower miscarriages rates."

Juergen Liebermann, Phd, HCLD, Director Laboratories, Fertility Centers, Illinois

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Vitrolife • Box 9080 • SE-400 92 Gothenburg • Sweden • Tel +46 31 721 80 00 • order@vitrolife.com • www.vitrolife.com