Collapsing of Blastocysts prior to vitrification

Joe Conaghan PhD

Pacific Fertility Center
San Francisco
USA

CRB Las Vegas
May 2013

Vitrification
Should we still be talking about it?

The Journal of
Clinical Embryology
Volume 16, Issue 1 • Spring 2013 ISSN 1941-1901

The Journal of Clinical Embryology™ *Volume 16, Issue 1*

Artificial Collapse prior Blastocyst Vitrification: Improvement of Clinical Outcomes

Juergen Liebermann, PhD, HCLD[1] and Joe Conaghan, PhD, HCLD[2]

[1]Fertility Centers of Illinois, Chicago, ILL.; [2]Pacific Fertility Center, San Francisco, CA.
Contacts: juergen.liebermann@integramed.com ; joe@pacificfertility.com

Collapsing blastocysts Isn't everybody doing it?

Message: 1
Date: Sun, 12 Aug 2012 11:30:47 +1000 (EST)
From: mbejds@hunterlink.net.au
Subject: EmbryoMail News - re Collapsing/Hatching of Vitrified Blastocysts.
To: em@embryomail.net
Message-ID: <56569.202.7.64.96.1344735047.squirrel@wm4.pacific.net.au>
Content-Type: text/plain;charset=iso-8859-1

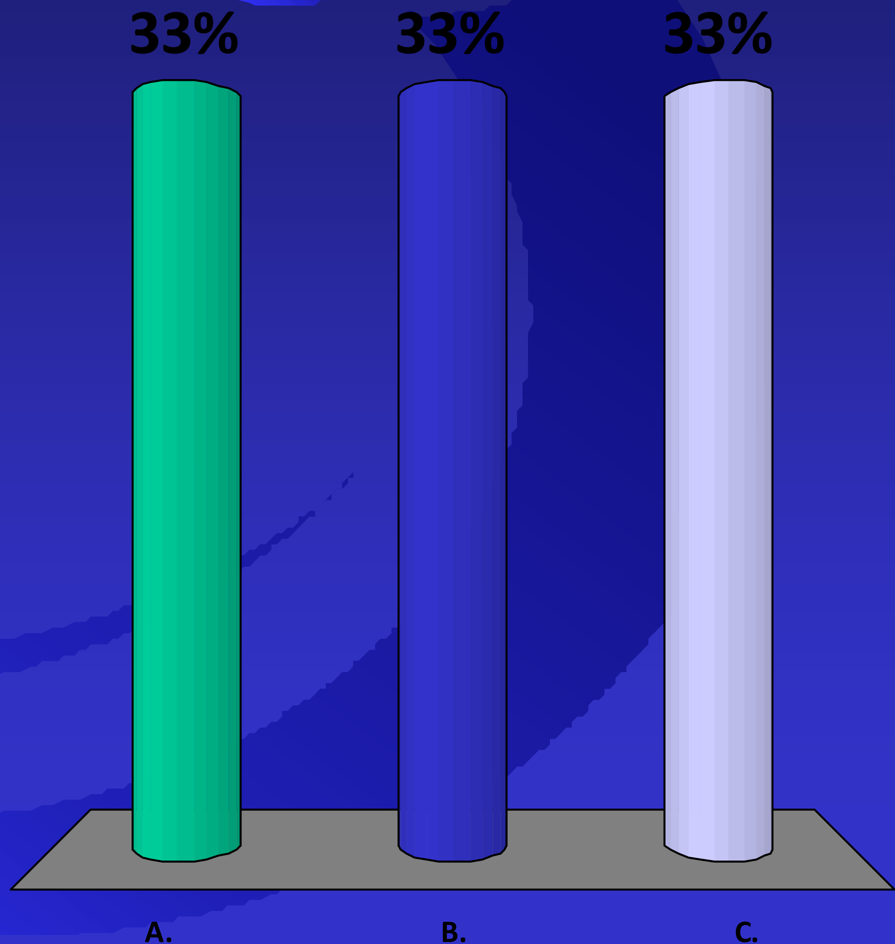
Re Collapsing and Hatching of Vitrified Blastocysts.
I have had a few enquires about hatching of vitrified blastocysts.
Recently I received by email via IRVIVE Scientific, a presentation of a ESHRE talk arguing the benefits of collapsing and hatching blastocyst by laser before and after warming. The link is at (http://www.fertaid.com/IVFDaily/IVFDaily.asp?sGetLink=2298).
There are only a few publications arguing for these techniques but acknowledge that Blastocyst biopsy is a form of combined collapsing/hatching. In my last clinic, we neither routinely collapsed nor hatched our vitrified blastocysts and were more than happy with the outcome. I am keen to know how prevalent these two interventions are in the industry and whether one or both may improve these rates even further.

Consequently I have created a 2 question survey on my FertAid Survey page (http://www.fertaid.com/fertaid/FertAid_Surveys.asp?SL=8&sOptions=B) and opened a blog (forum) at http://www.fertaid.com/IVFDaily/ClinicMail.asp?C=32&T=0 where anyone?s experience can be documented.
I am interested in hearing from anyone on their experiences either positive or otherwise. I will report back to Embryomail when it appears interest in this topic has waned.
I have no commercial interest in IRVINE Scientific but do with FertAid.

Dr James Stanger PhD
FertAid
Australia
office@fertaid.com

If you freeze excess blastocysts in your lab, do you….?

A. Freeze without artificial collapse
B. Manually collapse depending on stage or size of the blastocyst
C. Manually collapse all blastocysts before freezing



Special Offer for 2012
QAPonline $250

FertAid Surveys Return To List Replies Linked To IP Address12.208.210.3

Survey:Blastocyst Vitrification /True

Access: It is recommended that you logon or register to complete this survey. This will allow both an analysis by country and will improve accountability.You can submit answers linked to your IP address but they may not be utilised. REGISTER NOW

Comment: Many clinics routinely vitrify blastocysts but there is little information on how often the cavity is collapsed before vitrification and hatched after warming. This quick survey seeks to find out how common these techniques are.

1. **The Question:** Do you routinely collapse the blastocoel cavity before vitrification. **-Results [N=23]**

2. **The Question:** Do you routinely hatch the zona pellucida after warming the blastocyst? **-Results [N=25]**

Comments

Would You Like To Comment Further: You need to be registered to make a comment

Hum Reprod. 2003 Feb;18(2):384-91.

Vitrification of human blastocysts using cryoloops: clinical outcome of 223 cycles.

Mukaida T, Nakamura S, Tomiyama T, Wada S, Oka C, Kasai M, Takahashi K.

Hiroshima HART Clinic, 5-7-10 Ohtemchi, Naka-ku, Hiroshima, Osaka HART Clinic, Snowcrystal 10F 2-6-20, Umeda, Kita-ku, Osaka, Japan.

Abstract

BACKGROUND: The need to cryopreserve human blastocysts is increasing. The successful birth has been reported of a baby from a blastocyst vitrified using the cryoloop technique. The present study expands on this earlier report to confirm the effectiveness of this vitrification procedure.

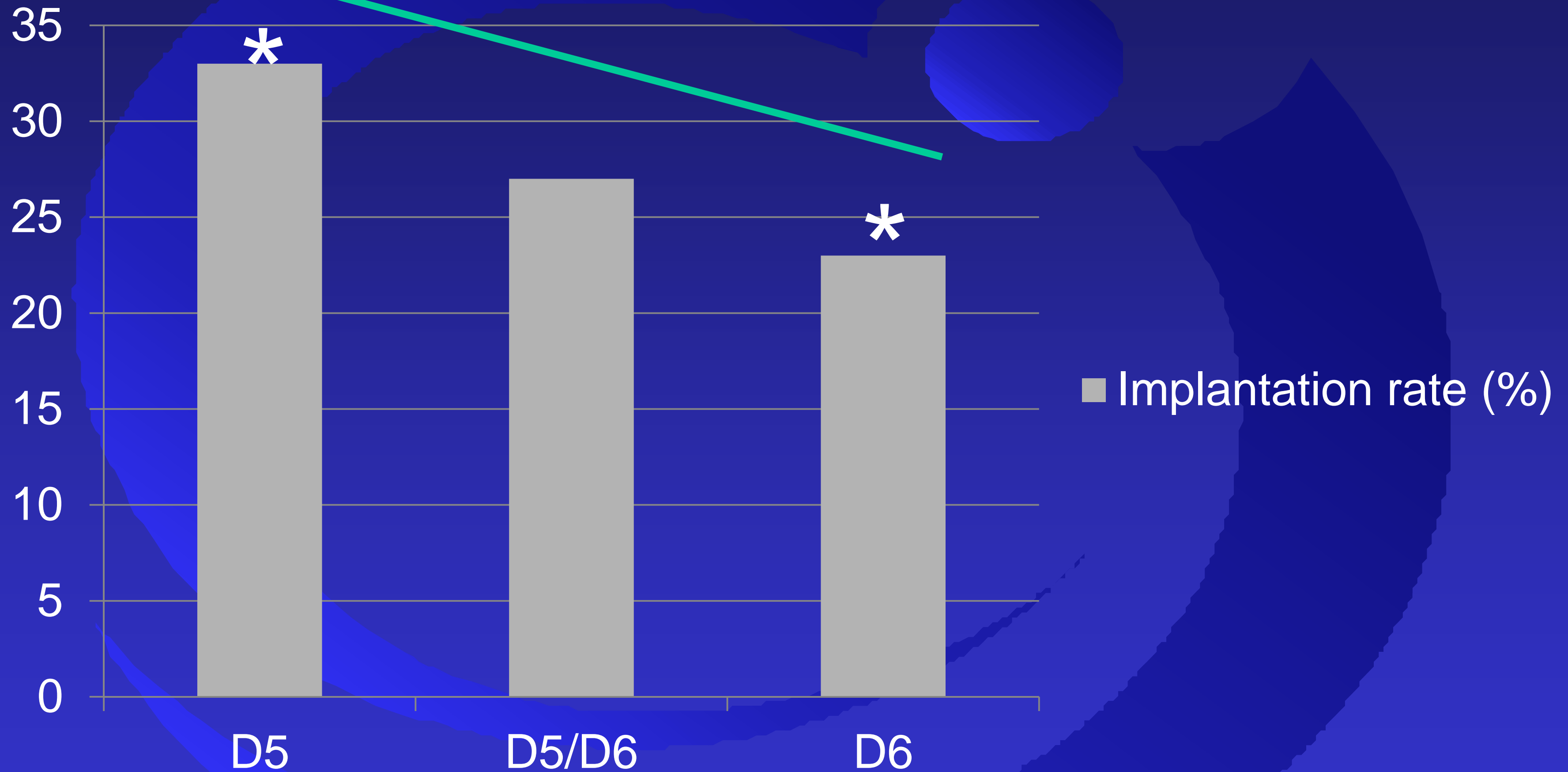
METHODS: In patients undergoing IVF at one of three clinics, supernumerary blastocysts on day 5 or 6 at various stages of development were vitrified using cryoloops.

RESULTS: Of 725 vitrified blastocysts, 583 (80.4%) survived. After the transfer of 493 blastocysts in 207 cycles, 76 women (37%) became clinically pregnant. Among these women, 21 pregnancies ended in miscarriage, 23 healthy babies were born in 18 deliveries, and 37 pregnancies are ongoing. The survival rate of day 5 blastocysts (87%) was higher than that of day 6 blastocysts (55%), but implantation rates and pregnancy rates were not statistically significantly different.

CONCLUSIONS: Clinical outcomes with 725 blastocysts and 207 transfers showed that vitrification using cryoloops is effective and practical for the cryopreservation of human blastocysts. Early blastocysts on day 5 seem to be the most suitable in terms of stage and age for cryopreservation, but developed and day 6 blastocysts can also be cryopreserved.

D5 and D6 differences

n=290, own oocytes

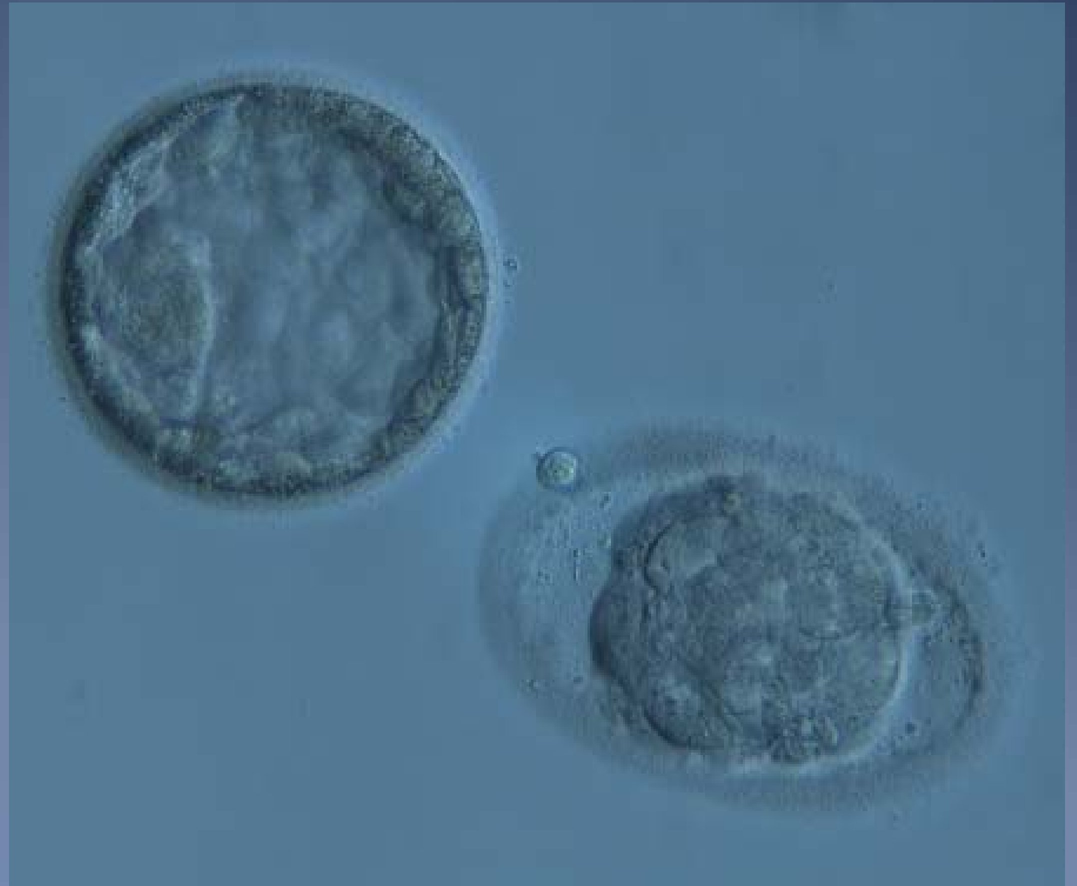


	D5 only	D5+D6	D6 only
Implantation/transfer	104/318 (33%)	29/107 (27%)	29/128 (23%)

* p= 0.03

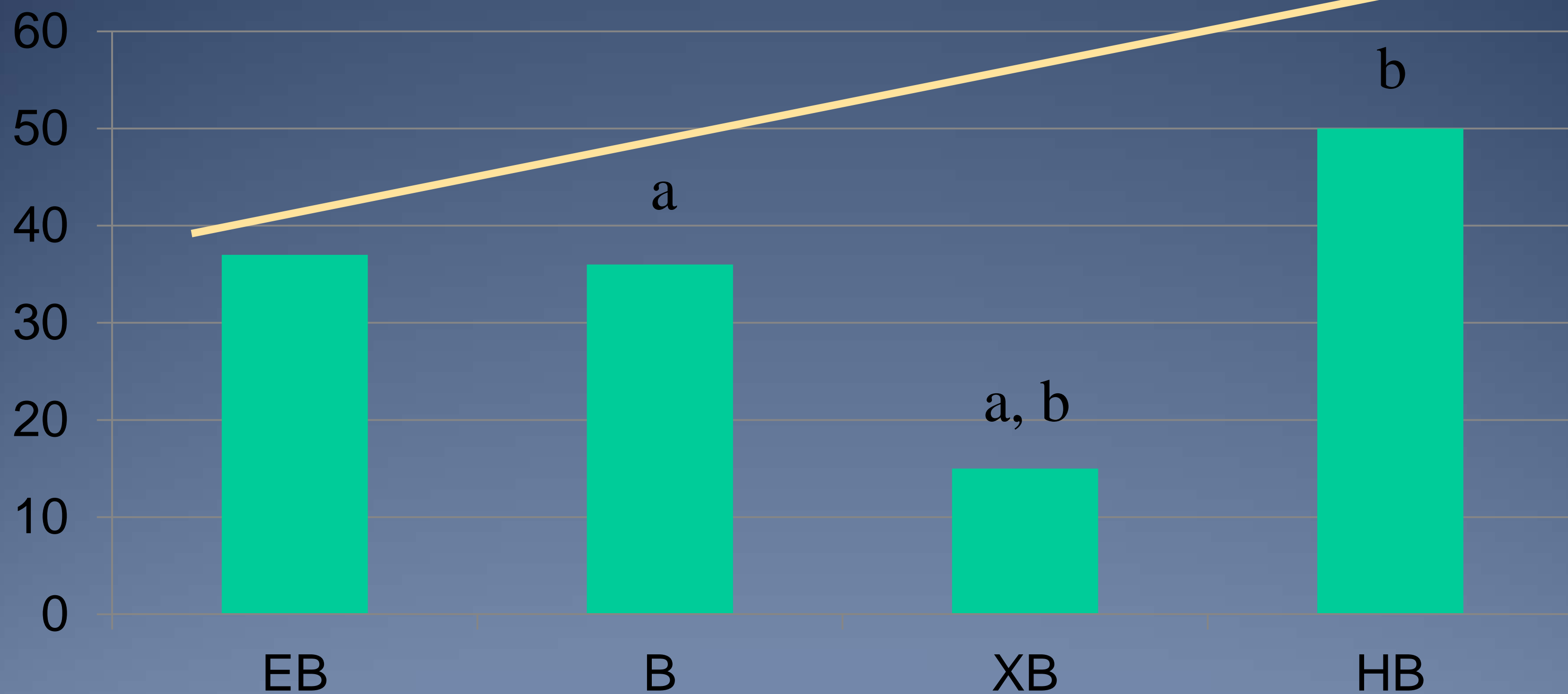
Warming expanded blastocysts

Embryos with a large cavity, that did not collapse on their own during vitrification, rarely survived warming!



Implantation by stage

SET only, n = 182



Early Blastocyst	Blastocyst	Expanded	Hatching
7/19	43/120	4/27	8/16

a, $p = 0.04$ and b, $p = 0.03$

Hum Reprod. 2002 Mar;17(3):744-51.

Births after vitrification at morula and blastocyst stages: effect of artificial reduction of the blastocoelic cavity before vitrification.

Vanderzwalmen P, Bertin G, Debauche Ch, Standaert V, van Roosendaal E, Vandervorst M, Bollen N, Zech H, Mukaida T, Takahashi K, Schoysman R.

Schoysman Infertility Management Foundation, Vaartstraat 42, 1800 Vilvoorde, Belgium. pierrevdz@hotmail.com

Abstract

BACKGROUND: In 1996, with the introduction of sequential media, we set up a programme of cryopreservation of supernumerary morulae (day 4) and blastocysts (day 5) using a vitrification procedure. Our results showed that the efficiency of the vitrification method was dependent on the stage of embryo development and was negatively correlated with the expansion of the blastocoele. We postulated that a large blastocoele might disturb cryopreservative potential due to ice crystal formation during the cooling step. We analysed therefore the effectiveness of reducing before vitrification the volume of the blastocoelic cavity.

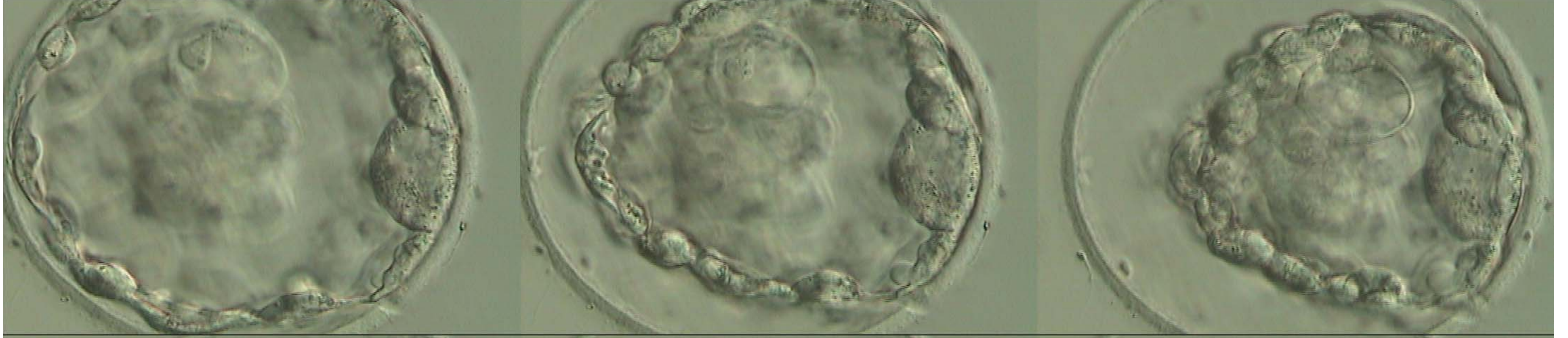
METHOD: Day 4 and day 5 embryos were vitrified in 40% ethylene glycol-18% Ficoll and 0.3 mol/l sucrose before plunging the straws directly into liquid nitrogen. Artificial shrinkage of the blastocyst was achieved after pushing a needle into the blastocoele cavity until it contracted.

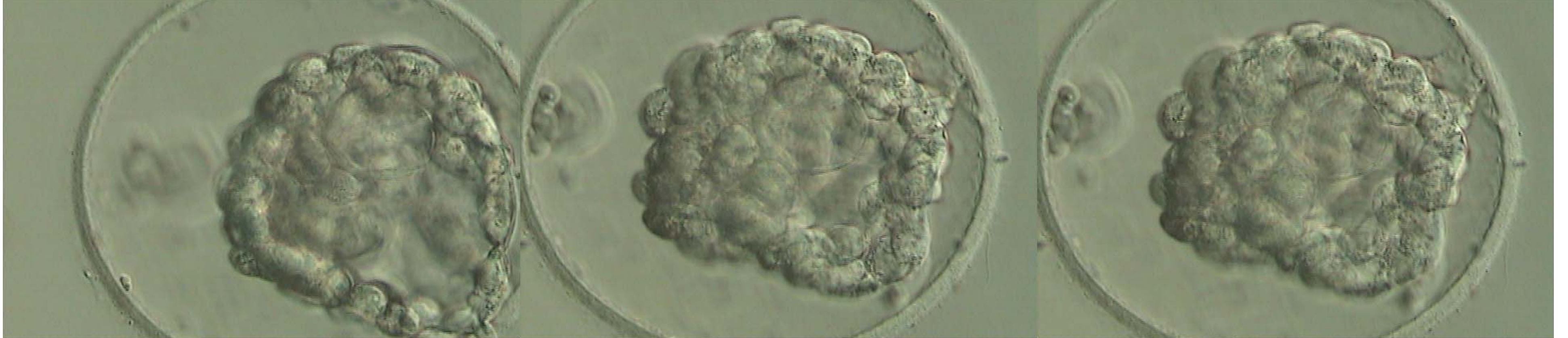
RESULTS: The survival rate post-thawing of day 4 and intact day 5 embryos was correlated with the volume of the blastocoele. In the control group only 20.3% blastocysts or expanded blastocysts survived as compared with 54.5 and 58.5% with morulae and early blastocyst respectively. After puncturing the blastocoelic cavity, an increase in the survival rate of up to 70.6% was noted. The pregnancy rates were improved after the artificial shrinkage procedure (20.5%) compared with the control intact blastocyst group (4.5%) (not significant). After reduction of the blastocoelic cavity, a significant increase in the implantation rate per vitrified blastocyst was observed (12.0 versus 1.4% $P < 0.01$).

CONCLUSIONS: Our results showed that survival rates in cryopreserved expanded blastocysts could be improved by reducing the fluid content. This was presumably because mechanical damage caused by ice crystal formation was avoided. These observations should be considered when establishing a strategy and a protocol for cryopreservation of day 5 embryos.

How to collapse a blastocyst

1. Laser: 1 x 450µs pulse on the junction between 2 trophectoderm cells
2. ICSI needle: Push needle through trophectoderm cell junction
3. Osmotic shock: Incubate blastocyst in 0.2 M sucrose solution for 1-2 minutes
4. Pipetting/needle: Physical manipulation with a small pipette or large needle
5. Biopsy: Blastocyst will collapse during trophectoderm biopsy





How to collapse a blastocyst

Laser: 1 x 450µs pulse on the junction between 2 trophectoderm cells

Fire a single shot

Wait (place embryo(s) back in incubator)

Have a cup of coffee

Transfer embryo into Equilibration Solution

Note: If you wait too long, the embryo may re-expand

J Assist Reprod Genet. 2011 Apr;28(4):355-61. Epub 2010 Dec 9.

In vitro and in vivo viability of human blastocysts collapsed by laser pulse or osmotic shock prior to vitrification.

Iwayama H, Hochi S, Yamashita M.

Yamashita Ladies' Clinic, Kobe, Hyogo, 651-0086, Japan. hiwayama@hotmail.co.jp

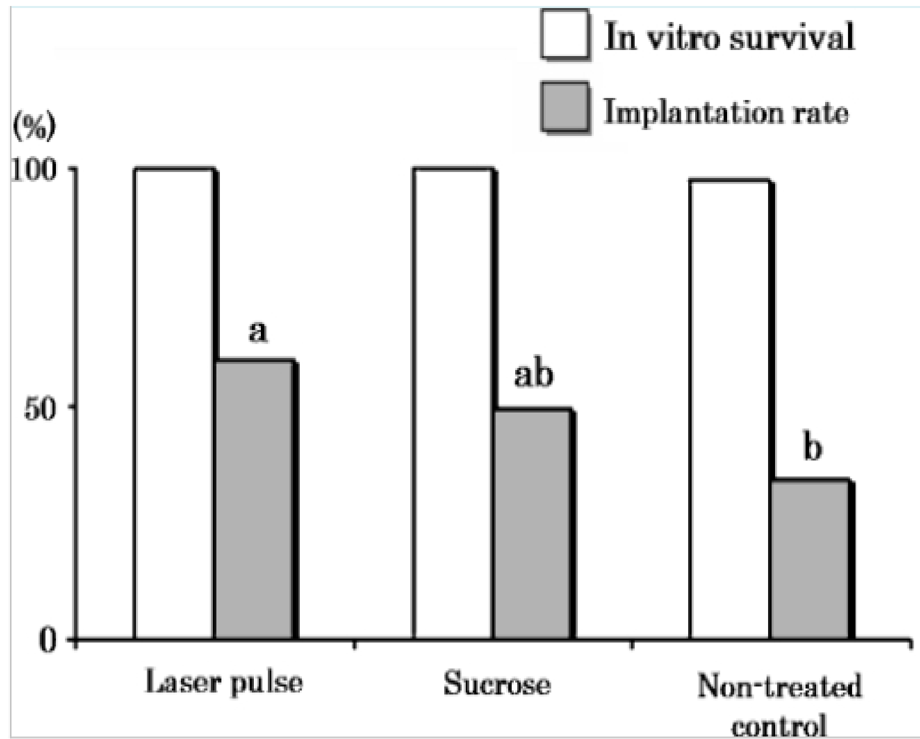
Abstract

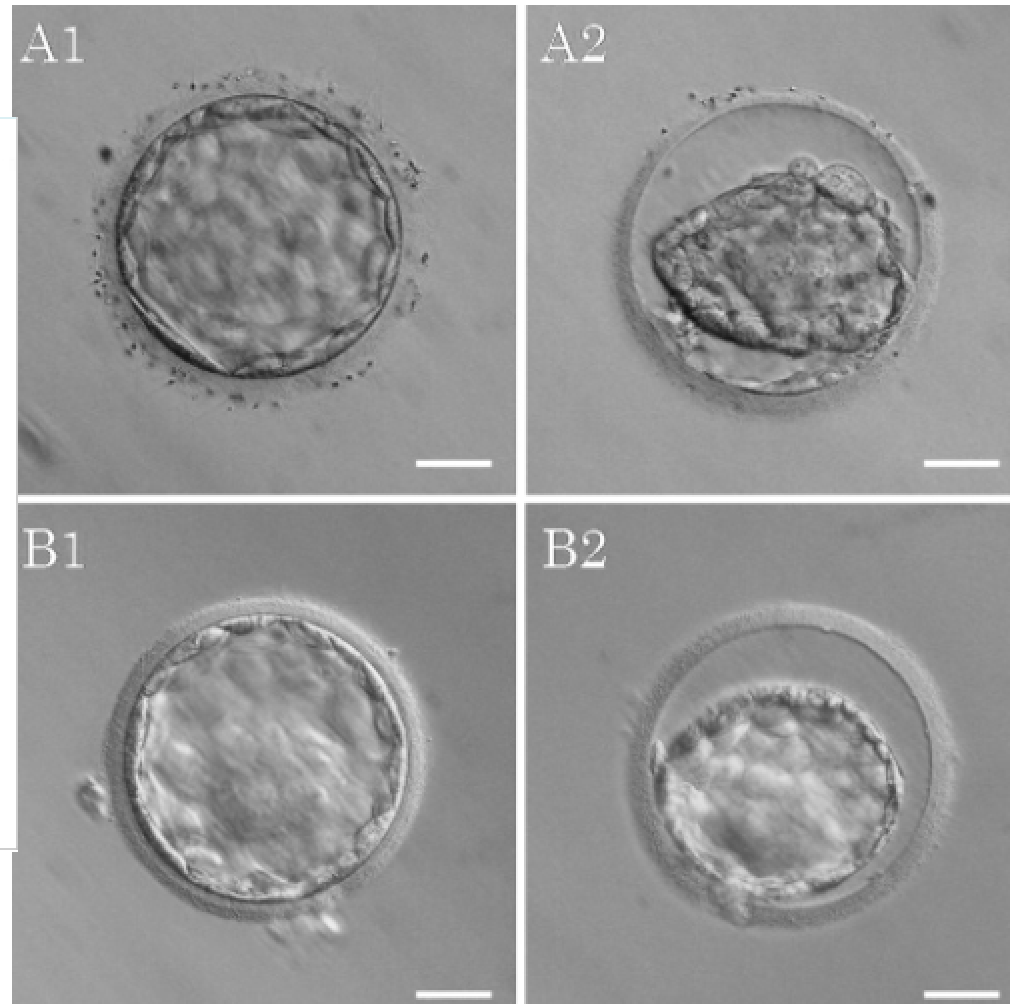
PURPOSE: This study was designed to investigate whether artificial shrinkage, induced by a laser pulse or hyperosmotic sucrose solutions, improves in vitro survival and/or implantation of vitrified-warmed human expanded blastocysts.

METHODS: Before Cryotop vitrification, the blastocoelic cavity was collapsed either by a laser pulse or sucrose solutions. Non-treated blastocysts were used as control. Post-warm blastocyst survival and implantation after transfer were examined. Implantation rate outcome was retrospectively analyzed by morphological grading and developmental kinetics of post-warm blastocysts.

RESULTS: Survival rates in the three groups were high. Implantation rates in the laser-pulse group (59.7%) were comparable with those in the sucrose group (49.3%), and were significantly higher than those in the control group (34.2%). The proportion of blastocysts showing fast development tended to be higher when the blastocysts underwent artificial shrinkage treatment before vitrification. There was no clear correlation between morphology of post-warm blastocysts and implantation rate.

CONCLUSION: Artificial shrinkage treatment before vitrification is associated with an increased probability of fast-developing embryos, resulting in higher implantation rates.





J Assist Reprod Genet. 2011 Apr;28(4):355-61. Epub 2010 Dec 9.

In vitro and in vivo viability of human blastocysts collapsed by laser pulse or osmotic shock prior to vitrification.

Iwayama H, Hochi S, Yamashita M.

Yamashita Ladies' Clinic, Kobe, Hyogo, 651-0086, Japan. hiwayama@hotmail.co.jp

How to collapse a blastocyst

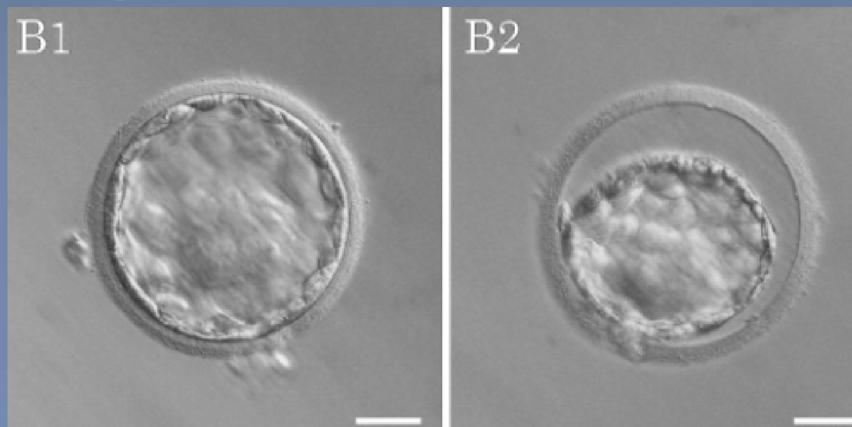
Osmotic shock: Incubate blastocyst in 0.2 M sucrose solution for 1-2 minutes

Make up 0.2M sucrose solution

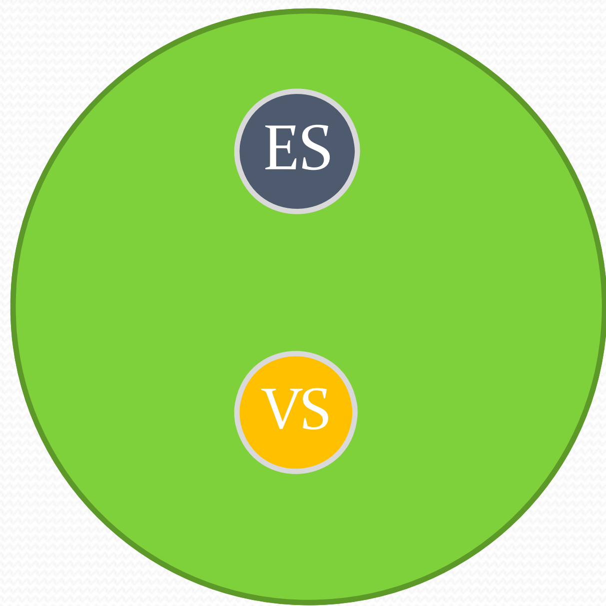
Place blastocyst in 0.2M sucrose

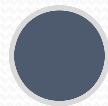
~~Have a cup of coffee~~

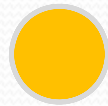
Move embryo to ES when >50% collapsed



Embryo vitrification dish setup



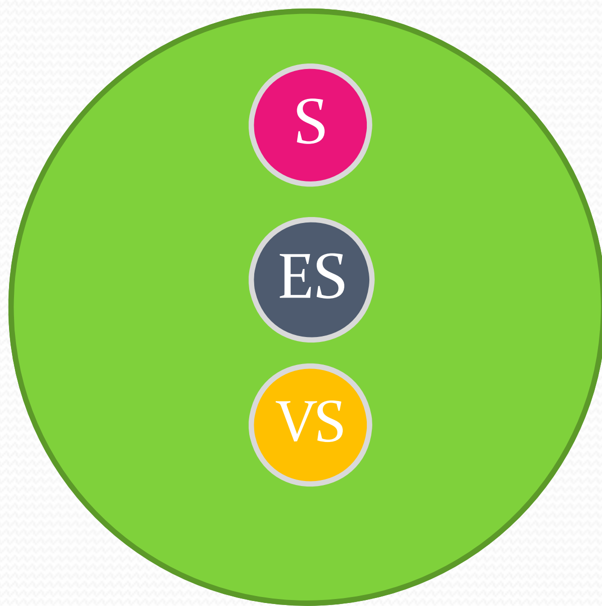
 50µl ES

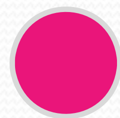
 50µl VS

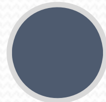
All at room temp.

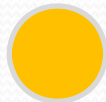
1. 8 mins in ES
2. 60-90 seconds in VS

Embryo vitrification dish setup




50µl Suc


50µl ES


50µl VS

All at room temp.

1. < 2 mins in 0.2 M Sucrose
2. 8 mins in ES
3. 60-90 seconds in VS

Theriogenology. 1996 Nov 1;46(7):1131-47.

Sucrose-induced shrinkage of in vitro produced bovine morulae: effect on viability, morphology and ease of evaluation.

Van Soom A, Ysebaert MT, Vanhoucke-De Medts A, Van de Velde A, Merton S, Delval A, Van Langendonckt A, Donnay I, Vanroose G, Bols PE, de Kruif A.

Department of Reproduction, Obstetrics and Herd Health, University of Gent, B-9000 Gent, Belgium.

Abstract

Sucrose (0.3 M) was used to cause artificial compaction of the embryonic cell mass of in vitro produced bovine embryos to facilitate morphological evaluation. Embryos were produced using routine in vitro maturation (IVM) and fertilization (IVF) techniques. The time necessary to induce shrinkage in 0.3 M sucrose to 75% of the original volume of Day 5 morulae was found to be less than I min, and 95% of the volume was regained in PBS after 2.5 min. No detrimental effect was observed after a 5- to 10-min sucrose treatment on subsequent blastocyst formation at Days 6 and 7 ($P > 0.05$). Furthermore, no significant differences were observed in the total number of cells, or in the mitotic and pycnotic cell index of blastocysts in different treatment groups. Agreement among 7 evaluators grading 40 Day 6 embryos was examined using the kappa coefficient of agreement (kappa). Overall agreement among evaluators for classification of quality grade was poor (48.2 %, kappa = 0.31) for embryos evaluated in PBS, but the rate improved when the same embryos were scored in sucrose (62.5 %, kappa = 0.49). Evaluating less compact in vitro produced bovine morulae in sucrose increases agreement among evaluators, since embryos in sucrose mimick the appearance of in vivo produced embryos. Thus, we conclude that scoring in vitro produced embryos in sucrose improves agreement among evaluators.

Hum Reprod. 2006 Dec;21(12):3246-52. Epub 2006 Aug 26.

Artificial shrinkage of blastocoeles using either a micro-needle or a laser pulse prior to the cooling steps of vitrification improves survival rate and pregnancy outcome of vitrified human blastocysts.

Mukaida T, Oka C, Goto T, Takahashi K.

Hiroshima HART Clinic, Naka-ku, Hiroshima, Japan. info@hiroshima-hart.jp

Abstract

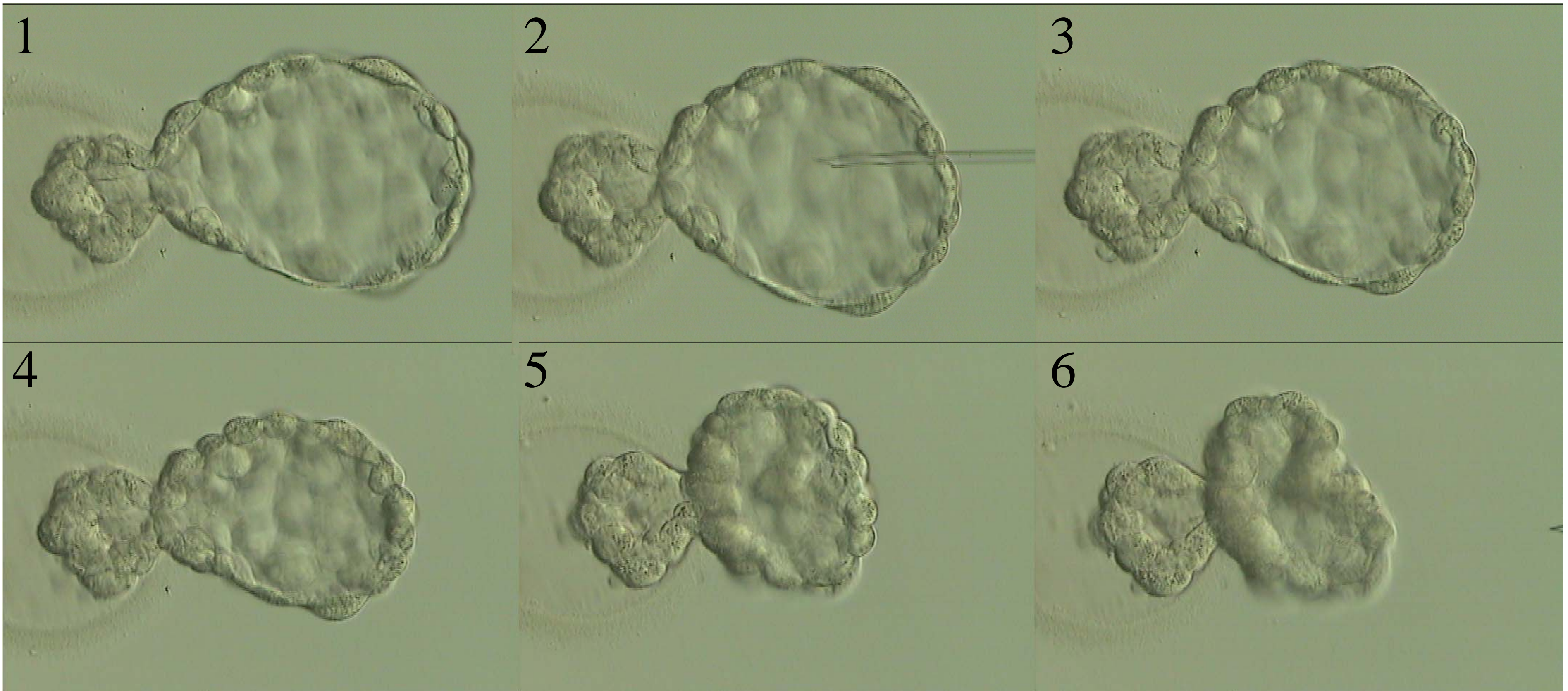
BACKGROUND: Since we reported the first successful birth from a blastocyst vitrified using a cryoloop technique, our results showed that the survival rate of vitrified blastocysts was negatively correlated with the expansion of the blastocoele. We speculated that a large blastocoele may disturb the efficacy of vitrification. Therefore, we evaluated the effectiveness of artificial shrinkage (AS) of blastocoeles before vitrification, on increasing the survival rate of vitrified blastocysts.

METHODS: Supernumerary expanded blastocysts on day 5 were vitrified after AS, which was performed by puncturing the blastocoele with a micro-needle, or by making a hole in the blastocoele with a laser pulse. After warming, viable blastocysts (confirmed by re-expansion of the blastocoele) were transferred to patients with hormone replacement cycle. We compared these data with those of our previous report where AS was not carried out.

RESULTS: The survival rate was significantly higher (97.2%, 488/502) in this study than that of the previous report (86%). After 266 transferable cycles, 160 patients became pregnant (60.2%), which was significantly higher than our previous results (34.1%, 29/85). The implantation rate was 46.7% (209/448).

CONCLUSIONS: Our results revealed that the survival rate and the pregnancy rate of vitrified expanded and hatching blastocysts can be improved by using AS to collapse the blastocele before vitrification.

Collapsing with an ICSI Needle



How to collapse a blastocyst

ICSI needle: Push needle through trophectoderm cell junction

Set up micromanipulator
Place blastocysts in ICSI dish
Puncture blastocysts between 2 TE cells
Jiggle the needle in the hole for 5-10 sec.
(or aspirate fluid)
Move embryos into Equilibration solution

Clin Exp Reprod Med. 2011 Jun;38(2):87-92. Epub 2011 Jun 30.

Effect of artificial shrinkage on clinical outcome in fresh blastocyst transfer cycles.

Hur YS, Park JH, Ryu EK, Yoon HJ, Yoon SH, Hur CY, Lee WD, Lim JH.

Maria Fertility Hospital, Seoul, Korea.

Abstract

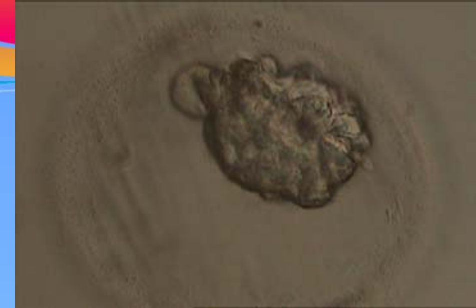
OBJECTIVE: This study aimed to determine the safety and clinical effect of artificial shrinkage (AS) in terms of assisted hatching of fresh blastocysts. Also, we evaluated the correlation between patient age and the effect of AS on clinical outcome.

METHODS: Two AS methods, using a 29-gauge needle and laser pulse, were compared. Seventy-three blastocysts were shrunk using a 29-gauge needle and the same number of other blastocysts were shrunk by a laser pulse. We evaluated the shrunken blastocysts hourly and considered them viable if they re-expanded >70%. Blastocyst transfer cycles (n=134) were divided into two groups: a control group consisted of the cycles whose intact embryos were transferred (n=100), while the AS group consisted of the cycles whose embryos were replaced following AS (n=34). The implantation and pregnancy rates of the control group and AS group were compared ($p<0.05$).

RESULTS: The re-expansion rates of the 29-gauge needle and laser pulse AS groups were similar (56 [76.7%] vs. 62 [84.9%], respectively). All of the remaining shrunken blastocysts were re-expanded within 2 hours. There was no degeneration of shrunken blastocysts. The total and clinical pregnancy rate of the AS group (23 [67.6%]; 20 [58.8%], respectively) was significantly higher than that of the control group (47 [47.0%]; 39 [39.0%], respectively). In the older patient group, there was no difference in the clinical outcomes between the AS and control groups.

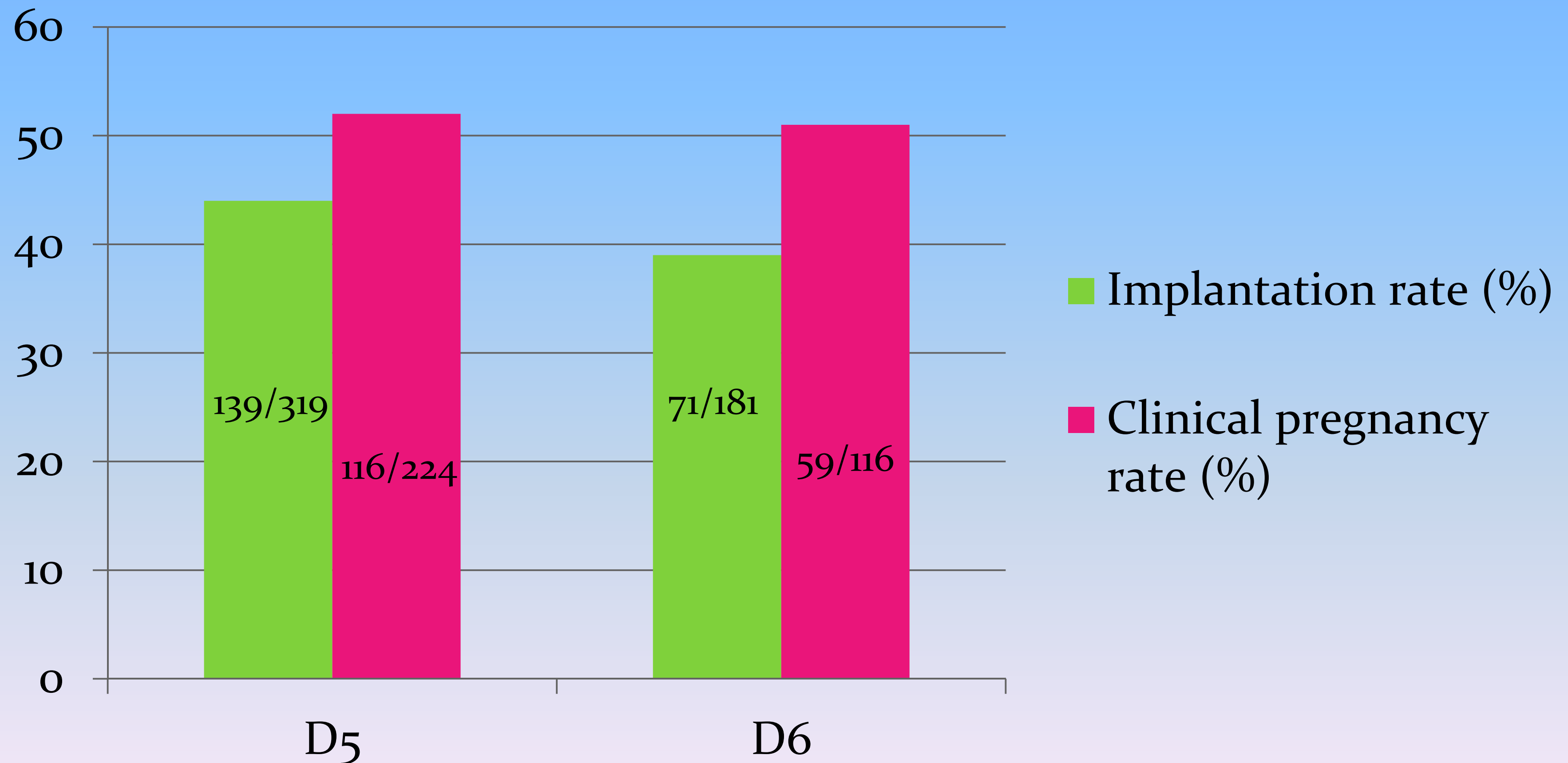
CONCLUSION: These results suggest that AS of blastocoele cavity, followed by the transfer, would be a useful approach to improve the clinical outcome in cycles in which fresh blastocyst stage embryos are transferred.

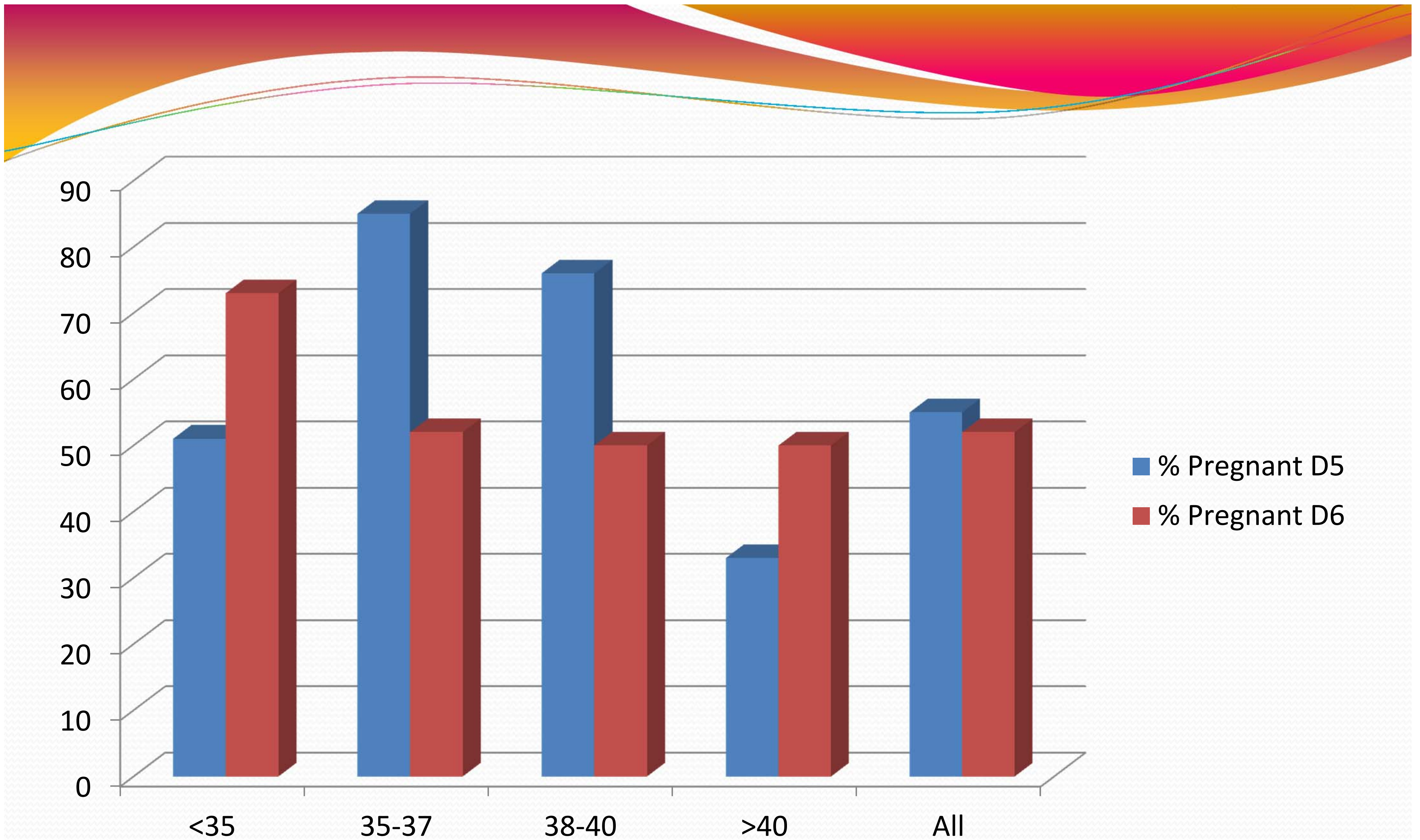
D5 and D6 Survival



* $p<0.05$

D5 and D6 differences in 2011





D5 (n=196) and D6 (n=123) pregnancy rates 2012

Result of introducing artificial collapse

1. Survival rates increased
2. Differences between implantation rates between D5 and D6 blastocysts went away
3. Pregnancy and implantation rates increased
4. Expanded blastocysts now implant at the expected rate
5. Embryologists refuse to warm embryos from old cycles

Game plan: Freezing

1. Aggressively vitrifying blastocysts
2. Fairly “loose” in what we will vitrify
3. Collapsing any blastocysts that we can
4. Only one embryo/straw
5. Results continuing to improve

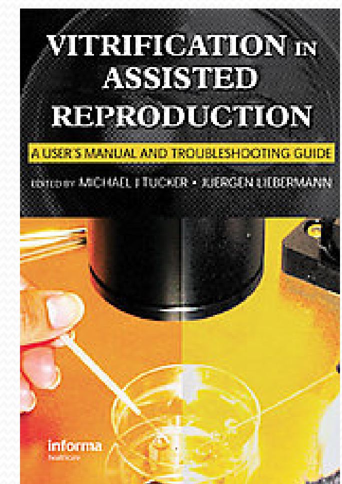
Game plan: Thawing

1. Aim is to thaw and transfer 1
2. Young patients, D5 embryos, collapsed
3. Thaw 30-60 mins prior to FET
4. Culture in 20% SSS post warming
5. Type of cycle not a concern

Summary

- Vitrified blastocysts implant at similar rates to fresh embryos
- Artificial Collapse is an important procedure to maximize survival of blastocysts
- Many options for collapse
- Try it!

- “*Technical footprint*”
- “*Find what works for you*”



Acknowledgements

- Erin Fischer
- Jean Popwell
- Simona Torcia
- Sergio Vaccari
- Valerie Hines
- Jennifer Andres
- Arianna Conti
- Amy Kittleson

joe@pacificfertility.com

©2013 by the College of Reproductive Biology (CRB). All rights reserved.