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ARTICLE

Vitrification of human embryos previously cryostored by either slow freezing or vitrification results in high pregnancy rates

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Abstract Occasionally, clinical scenarios arise where embryos, previously cryostored and warmed, need to be recryopreserved. The outcome of 30 such transfer cycles from 25 women where embryos were recryopreserved is detailed. In 16 cases, embryos were initially cryopreserved by slow freezing and in 14 cases by vitrification. The cryopreservation stages were the pronuclear stage (n = 16), day-3 cleavage stage (n = 12), blastocyst (n = 1) and oocytes (n = 1). All recryopreservation was by Cryotop-based vitrification. From this mixed source, 30/31 twice-cryopreserved embryos survived warming and were transferred, resulting in 13 pregnancies, 11 deliveries with normal gestational age and birthweight, one pre-term birth at 33 weeks and two miscarriages. There were no malformations reported for the live births. Recryopreservation using vitrification by CryoTop has been used in a variety of clinical scenarios to preserve surplus cryopreserved embryos. The current study, although limited in numbers, resulted in high survival rates, clinical pregnancy rates similar to once-cryopreserved embryos and healthy live births independently of the initial stage and cryopreservation method. The technique may increasingly be applicable to elective single-embryo transfer and blastocyst transfer to maximize the pregnancy rate while minimizing the number of cryopreserved embryo transfers.

KEYWORDS: blastocysts, cryopreservation, oocytes, revitrification, slow freezing, vitrification

Introduction

Recryopreservation is a powerful tool for the embryologist. The ability to vitrify and warm embryos multiple times allows patients to micromanage their cryostored embryos. Recent trends in IVF towards single-embryo transfer and blastocyst transfer create scenarios where recryopreservation may be a useful tool for clinicians and scientists in recommending various treatment options to couples. As an increasing number of cycles have only a single embryo transferred, more embryos are becoming available for cryopreservation. If the embryos are cryopreserved at the blastocyst

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stage, then rarely will recryopreservation be appropriate. However, many clinics do not culture all embryos to the blastocyst stage because of the uncertainty in the number of day-5 embryos that will be available or because of reduced embryo quality. Where numerous embryos are cryopreserved at the cleavage stage, couples may be faced with decisions in how to use the cryopreserved embryos, either by repeated cleavage-stage transfers or by bulk warming and extended culture to identify the best embryo for transfer. If after culture, surplus good-quality embryos remain, these may be recryopreserved. The ability to successfully recryopreserve surplus good-quality embryos allows the patient to explore such options to maximize their chances of pregnancy with minimal treatment. Knowing that healthy embryos are not wasted is a key parameter in their decision-making process and a valuable option for clinicians when discussing treatment options. Other scenarios reported here include cases where embryo transfer needs to be rescheduled after embryo warming and when oocytes rather than embryos have previously been cryopreserved. The later situation may become more common as oocyte cryopreservation activity increases.

When occasional embryos have been refrozen and thawed (Baker et al., 1996) using conventional protocols (Testart et al., 1986), there has been cryoinjury sufficient to largely preclude its widespread employment. Vitrification, at least in the animal models, allows for multiple freezing with minimal damage (Sheehan et al., 2006) and there may be an opportunity for it to be applied in a clinical environment. There have been several recent reports detailing successful revitrification, including embryos (Kumasako et al., 2009), oocytes (Chang, 2008) and after biopsy (Peng et al., 2011); however, these are isolated cases. This article reports on the outcome of 30 cases where embryos have been revitrified after an initial freezing/vitrification event.

Materials and methods

A total of 25 couples had 30 treatment cycles involving the transfer of embryos that had initially been cryopreserved by either slow freezing or vitrification and subsequently revitrified. The details of patient demographics, embryology and outcomes of the cycle in which embryos or oocytes were initially cryopreserved are presented in **Table 1**. Nine couples were aged <35 years, nine were aged 35–39 and seven were aged ≥40 years. Three cases involved donated embryos and one case involved donated oocytes that had been cryopreserved as oocytes before warming, followed by insemination and revitrification of surplus embryos. The patient demographics and the basic embryology in the original IVF cycle in the couples with revitrification were similar to all other cases involving the transfer of vitrified embryos in 2009–2010 (**Table 2**), although the number of oocytes and embryos were higher in the study group.

There were 10 cases where all embryos had been cryopreserved due to risk of ovarian hyperstimulation and two cases where the original IVF embryo transfer produced a live birth and the couples, returning for a second pregnancy, elected to warm and culture all the cryopreserved embryos to identify the most viable embryo for transfer. These bulk warmings resulted in surplus embryos that were recryopreserved. Two cases involved recryopreservation of the embryos on the same day as the warming: the first where the embryo transfer was cancelled after the slow-frozen embryo had been thawed and the embryos were vitrified 2 h later; the second where the client had wished all her vitrified blastocysts to be warmed, one to be transferred and the balance discarded with the intention of ceasing all further treatment – one surplus embryo was of sufficient quality to be cryopreserved and the client changed her management and requested it to be revitrified. While most of the initial warming cycles involved relatively large numbers of embryos with a view towards embryo selection (i.e. including extended culture to identify the best embryo), all recryopreserved–warmed embryos were transferred as single embryos.

Some initial cryopreservation was by slow freezing but all recryopreservation was by vitrification on either day 3 or day 5/6. Sixteen cases had embryos initially cryopreserved using conventional propanediol/sucrose protocols (Testart et al., 1986) and 14 cases were initially vitrified by the CryoTop method as described by Kuwayama et al. (2005a,b). In 16 cases, embryos were initially cryopreserved at the pronuclear stage, in 12 cases at the day-3 stage, in one case as blastocysts and in one case as oocytes (**Table 1**). The same vitrification procedure was employed for cleavage- and blastocyst-stage embryos and the initial and second cryopreservation events. Media was prepared fortnightly and tested for quality using immature oocytes which were not subsequently used after IVF. Each media batch required a survival rate >90% for approval for clinical use.

The vitrification protocol used preparations based on M199 media (Sigma, Australia) and supplemented prior to use with either 10% heat-inactivated patient serum or 10 mg/ml human serum albumin (HSA; Sage Biopharma, Gytech, Australia) if serum was unavailable. Embryos were held at 37°C in cleavage media until vitrification. Each embryo was individually vitrified by transferring to initial cryosolution containing 7.5% dimethylsulphoxide (DMSO; Sigma), 7.5% ethylene glycol (Sigma) in M199 plus serum/HSA for 15 min at room temperature before transfer to the final cryosolution (15% DMSO, 15% ethylene glycol in M199 plus serum/HSA) also at room temperature. The embryos were held in the final solution for 50 s before being rapidly loaded onto the CryoTop tool and excess cryoprotectant was removed to leave a fine meniscus of fluid over the embryo before plunging into virgin liquid nitrogen. The tool was then encased in the protective sheath before cryostorage. The warming process involved very rapid transfer to the first solution (1 mol/l sucrose (Sigma) for 50 s at 37°C, followed by 3 min in the dilution media (0.5 mol/l sucrose) at room temperature then 5 min in washing solution (no sucrose) also at room temperature. The embryos were then warmed in a HD Scientific Work Station before washing in cleavage (PN stage) or blastocyst media (day-3 or day-5 stage; Sage). Embryo culture was in 10 μl cleavage/blastocyst media under mineral oil at 37°C in MINC incubators (Cook, Australia) with 6% CO_2, 5% O_2 and nitrogen balance. Prior to transfer, a day-3 embryo or blastocyst was moved to transfer solution, containing blastocyst culture media enhanced with 10% HSA (Sage) before embryo transfer in 10–20 μl fluid. When the transfer was on the same day as the warming,

Table 1 Initial cryopreservation method of oocytes/embryos, patients' age, fertility history, embryology and IVF outcome by embryo transfer day.

ET day	*Initial cryopreservation*	*Age (years)*	*History*	*Embryology*[a]	*IVF outcome*
3	Vitrification (2PN)	36	POF, MF, donor spermatozoa	11/10/0/10	No ET
3	Vitrification (2PN)	43	Ovum recipient[b]	9/7/0/7	No ET
3	Vitrification (oocyte)	40	Azoospermia, OF	6/4/1/1	NP
3	Slow freeze (2PN)	27	POF	Donated	NA
3	Slow freeze (2PN)	37	Uterine/vasectomy	11/7/1/4	Live birth
3	Slow freeze (2PN)	44	Uterine	Donated	NA
3	Vitrification (D3)	32	PCO, MF	18/7/1/3	NP
5	Vitrification (D5)	36	Endometriosis	7/5/2/3	Live birth
5	Vitrification (2PN)	25	PCO, MF	21/14/0/14	No ET
5	Vitrification (2PN)	31	PCO	23/11/1/4	NP
5	Slow freeze (2PN)	45	Endometriosis	17/12/2/4	NP
5	Slow freeze (2PN)	35	PCO, MF	18/14/0/14	No ET
5	Slow freeze (2PN)	31	PCO, MF	25/13/0/13	No ET
5	Slow freeze (2PN)	42	Uterine	12/8/0/8	No ET
5	Slow freeze (2PN)	40	Ovum recipient	4/4/1/2	NP
5	Vitrification (2PN)	43	MF	6/5/0/5	No ET
5	Slow freeze (2PN)	37	Endometriosis	Donated	NA
5	Vitrification (2PN)	37	PCO, MF	20/13/0/13	No ET
5	Slow freeze (2PN)	35	PCO, MF	16/15/0/15	No ET
5	Slow freeze (D3)	30	Donor spermatozoa	Imported	NP
5	Vitrification (D3)	30	Donor spermatozoa	8/5/1/4	NP
5	Slow freeze (D3)	35	Tubal	19/16/0/16	No ET
5	Slow freeze (D3)	37	Tubal, MF	16/12/1/8	NP
5	Slow freeze (D3)	25	PCO, MF	11/8/1/4	NP
5	Slow freeze (D3)	31	Unexplained	14/12/1/9	NP

2PN = two pronuclei; D = day; donor spermatozoa = donated spermatozoa used for insemination; ET = embryo transfer; MF = male factor; NA = not applicable; NP = not pregnant; OF = oocyte freezing; PCO = polycystic ovaries; POF = premature ovarian failure; Uterine = fibroids and other uterine abnormalities.
[a]Embryology = oocytes, embryos, transferred, cryopreserved.
[b]Donated oocytes had been cryopreserved by vitrification as oocytes.

Table 2 Patient demographics and original embryology in the revitrification group and routine vitrification cycles.

	Woman's age (years)			
	Revitrification		*Routine vitrification*	
	<35	*≥35*	*<35*	*≥35*
Infertility				
Tubal	0	2	27	38
Endometriosis	0	2	32	40
Male factor	6	6	110	151
Unexplained	1	0	82	116
Other	2	5	89	166
Total	9	16	340	511
Embryology				
Oocytes	16.8 ± 6.3	12.4 ± 5.5	11.1 ± 5.6	10.9 ± 4.9
Embryos	11.1 ± 3.5	9.1 ± 4.2	7.1 ± 3.9	6..5 ± 4.1
Embryos cryopreserved	9.0 ± 5.1	6.9 ± 4.6	5.9 ± 3.2	4.8 ± 3.5

the time between warming and transfer varied between 0.5 and 3 h. Pronuclear-stage embryos were cultured in cleavage-stage media (Sage) and transferred either 2 or 4 days later. Standard protocols existed for extended culture.

All transfers were under a hormone replacement treatment (HRT) regimen. Essentially, the HRT regimen started with 6 mg oestradiol valerate tablets (Progynova, 2 mg t.i.d.; Schering Plough) from day 1 of the menstrual cycle.

The dose was reviewed on cycle day 10 and increased to 12 mg (4 mg t.i.d.) if the oestradiol concentration was <1000 pmol/l, and continued for at least a further 5–7 days. Once serum oestradiol was greater than 1000 pmol/l, women were given 20 mg vaginal oestradiol pessaries (PIVET formulation, containing 20 mg micronized 17β-oestadiol in a standard fatty acid base) for 5 days in addition to oestradiol valerate. Day-10 endometrial thickness measurement was performed in all women by 3D ultrasound (General Electric, Voluson). After 5 days, oestradiol concentrations and endometrial thickness were checked to ensure endometrial thickness ≥8 mm. If endometrial thickness persisted in being <8 mm, and particularly if the uterine fundal sagital area was <15 cm^2 or if the uterine volume was <25 ml, the oestrogen regimen could be continued, sometimes increasing the vaginal pessary dosage to 20 mg b.i.d.

Once endometrial thickness measured ≥8 mm, the oestrogen pessaries were ceased and replaced with progesterone, designating the onset of the luteal phase of the HRT cycle. The regimen involved continuing oestradiol valerate at the final defined dose, described above, while the oestradiol pessaries were replaced with progesterone pessaries 400 mg b.i.d. and oestradiol/progesterone at night. The progesterone pessaries were produced by a local pharmacy to PIVET specifications and tested for sperm toxicity by the sperm survival assay. The pessaries were prepared using 400 mg progesterone in a fatty acid base and the oestradiol/progesterone-combined pessaries prepared as 500 mg micronized progesterone and 2 mg miconized 17β-oestradiol in the same fatty acid base. All women had mid-luteal oestradiol and progesterone concentrations checked on day 8 of progesterone administration and the above doses could be increased if the oestradiol was <1000 pmol/l or the progesterone was <40 nmol/l. The oestradiol valerate tablet/progesterone pessary regimen was continued to day 16 of the luteal phase, when the β-human chorionic gonadotrophin concentration was measured; pregnancy was diagnosed at 50 IU/l and later confirmed by the presence of a uterine sac. If pregnancy was identified, the HRT regimen was continued until week 12 of pregnancy. Biochemical pregnancies were noted but not included in the pregnancy rate.

Embryo transfers were conducted on either day 4 or 6 of progesterone in the lithotomy position with moderate Trendelenberg tilt (head down) under ultrasound guidance. Depending upon the uterine position (anteverted, axial or retroverted), the bladder was allowed to fill to the degree that enabled a satisfactory transvesical ultrasonic view of the endometrial cavity. The single day-3 embryo or blastocyst was transferred utilizing the K-JETS catheter system (K-Jets-7019-SIVF; Cook) and a clear mid-fundal flash was identified on ultrasound, signifying an appropriately conducted embryo transfer.

The survival and clinical pregnancy rates of recryopreserved embryos were compared with the routine vitrification transfers that occurred during 2009 and 2010, the time frame when the recryopreserved embryos were thawed. The comparison was between women <35 years and ≥35 years of age and by transfer stage.

All aspects of embryo vitrification, including revitrification, were approved as an innovative procedure by the Reproductive Technology Council, Western Australia in 2007 (approval I-023) not requiring separate ethical approval.

Results

This study reports on 29 cases where cryopreserved embryos were warmed and then revitrified for a variety of reasons and one case where oocytes that had been vitrified were warmed, inseminated and the resultant embryos cultured to day 3. In this case, four oocytes were warmed, three embryos were created, one day-3 embryo was transferred and one surplus day-3 embryo was recryopreserved. In all, 31 recryopreserved embryos were warmed; of these, 30 survived and were transferred. All cleavage-stage embryos (16/16) and 14/15 blastocysts survived recryopreservation and were transferred. In the case where one blastocyst did not survive warming, a second blastocyst was warmed and the transfer proceeded as planned. These rates were similar to that achieved from the routine vitrification programme (**Table 3**). Although the original number of oocytes and embryos were higher in the study group than in the concurrent patients having embryos vitrified, it is unlikely that this would have influenced embryo survival.

When the revitrified embryos were subsequently warmed in new transfer cycles, 13 clinical pregnancies resulted from the 30 transfers (**Table 4**). Seven pregnancies arose after the embryos, initially cryopreserved at the pronuclear stage, were warmed, cultured and revitrified on either day 3 (3/7) or day 5 (4/13). Five pregnancies arose from embryos initially cryopreserved as day-3 embryos and revitrified on day 3 (1/1) or cultured to day 5 and then revitrified (4/7). One pregnancy arose from a number of surplus day-5 embryos that had initially been vitrified, warmed (with a view to being discarded) but then revitrified and warmed in a subsequent cycle (1/1). Both women for whom the embryos were recryopreserved on the same day as warming conceived after the transfer of the revitrified embryo.

There were four pregnancies from 11 transfers in women aged <35 years and nine pregnancies from 19 transfers in women aged ≥35 years. These results were similar to the current clinical pregnancy rate for transfers performed between 2009 and 2010 from vitrified embryos, partitioned by age and stage at vitrification (**Table 5**). This observation implies that a second cryostorage event had no impact on the embryo viability, recognizing that the embryos suitable for recryopreservation were, by definition, healthy.

Eleven of the 13 clinical pregnancies were viable. Two resulted in a miscarriage at 8 and 9 weeks of a sac without a fetal heart. A further biochemical pregnancy was not included in the pregnancy rate. There were 11 live births, all singleton deliveries of normal gestational age and birthweight except one pre-term delivery at 33 weeks. Eight of the deliveries were males and three were females (**Table 6**). There were no malformations reported for these births.

Discussion

The capacity to revitrify embryos provides the patient and the clinic with significant advantages by maximizing the chance of pregnancy while minimizing the number of transfers. This approach sees the sum of fresh and cryopreserved

Table 3 Survival rates of revitrified embryos compared with routine vitrification–warming.

Embryo age at revitrification	*Recryopreserved*	*Routine vitrification–warming*[a]
Day 3	16/16 (100)	129/173 (75)
Day 5/6	14/15 (93)	69/77 (90)
Total	30/31 (97)	198/250 (79)

Values are *n*/total (%).
[a]Warmed embryos vitrified between 2009 and 2010.

Table 4 Clinical pregnancy rates according to the day of initial cryopreservation and stratified by age.

Cryopreservation stage		*Clinical pregnancies/transfer*		
Initial	*Recryopreservation*	*<35 years*	*≥35 years*	*Total*
Oocyte	Day 3	–	0/1 (0)	0/1 (0)
2PN[a]	Day 3	0/1 (0)	3/6 (50)	3/7 (43)
2PN	Day 5	0/3 (0)	4/10 (40)	4/13 (31)
Day 3[b]	Day 3	1/1 (100)	–	1/1 (100)
Day 3	Day 5	3/6 (50)	1/1 (100)	4/7 (57)
Day 5[b]	Day 5	–	1/1 (100)	1/1 (100)
Total		4/11(36)	9/19 (47)	13/30 (43)

Values are *n*/total (%). 2PN = two pronuclei.
[a]There was one additional biochemical pregnancy.
[b]Initial embryo transfer cancelled after embryos had been warmed.

Table 5 Comparison of clinical pregnancy rates for recryopreserved embryos and routine vitrification stratified by age and day of transfer.

ET day	*Woman's age (years)*					
	Recryopreserved			*Routine vitrification*[a]		
	<35	*≥35*	*Total*	*<35*	*≥35*	*Total*
Day 3	1/2 (50)	3/7 (43)	4/9 (44)	34/99 (34)	51/197 (26)	85/296 (29)
Day 5	3/9 (33)	6/12 (50)	9/21 (43)	41/92 (45)	49/118 (42)	90/210 (43)
Total	4/11 (36)	9/19 (47)	13/30(43)	75/191 (39)	100/315 (32)	175/506 (35)

Values are *n*/total (%).
[a]All transfers between 2009 and 2010.

embryos as the end point of a treatment cycle and cumulative pregnancy rates as more important than rates per fresh transfer (De Neubourg et al., 2010; Lundin and Bergh, 2007). Management of cryopreserved embryos is therefore an important component in the IVF cycle influencing drug regimens, and the ability to recryopreserve embryos is a valuable part of this process. This has become increasingly important with single-embryo transfers now dominating fresh transfers in younger women (De Neubourg et al., 2010). Embryos, of course, may be cryopreserved on any day between day 1 and day 6, depending on clinical circumstances. Many clinics are reluctant to routinely culture all embryos to the blastocyst stage because of the uncertainty of outcome and because part of the management strategy for a treatment cycle is to have one embryo for transfer and surplus embryos for cryopreservation. Only when there are sufficient good-quality day-3 embryos to provide reasonable confidence that at least one embryo will be available for transfer is extended culture recommended to the patients. Also, but rarely these days, all embryos may be cryopreserved due to ovarian hyperstimulation syndrome. However, when the patient returns for a cryopreserved embryo transfer, they may elect to have individual embryos transferred over multiple cycles or to warm multiple embryos and extend their culture to help to identify the best embryo for transfer. The advantage in warming many embryos and extending their culture is that it allows the patient to minimize the number of potential transfers while maximizing their chance of pregnancy. The problem with this option occurs when there is more than one good-quality

Table 6 Clinical outcomes from recryopreserved embryos.

Transfer day	*Age (years)*	*Initial freeze*	*Outcome*	*Birth details*
3	44	Slow freeze (2PN)	Live birth (39 weeks)	Male (3590 g)
3	36	Vitrification (2PN)	Miscarried (9 weeks)	1 non-viable sac
3	43	Vitrification (2PN)	Miscarried (8 weeks)	1 non-viable sac
3	32	Vitrification (D3)	Live birth (39 weeks)	Male (3100 g)
5	32	Vitrification (D5)	Live birth (39 weeks)	Female (4020 g)
5	35	Slow freeze (2PN)	Live birth (39 weeks)	Male (3770 g)
5	37	Slow freeze (2PN)	Live birth (38 weeks)	Male (2551 g)
5	37	Vitrification (2PN)	Live birth (33 weeks)	Female (2055 g)
5	35	Slow freeze (2PN)	Live birth (39 weeks)	Male (3600 g)
5	30	Slow freeze (D3)	Live birth (38 weeks)	Male (4110 g)
5	37	Slow freeze (D3)	Live birth (37 weeks)	Male (3112 g)
5	25	Slow freeze (D3)	Live birth (41 weeks)	Female (3184 g)
5	25	Slow freeze (D3)	Live birth (39 weeks)	Male (3374 g)

2PN = two pronuclei; D = day.

blastocyst after extended culture: naturally, patients will be reluctant to waste embryos. However, the capacity to warm multiple embryos, select the 'best' embryo for transfer and recryopreserve the embryos that also continued to divide is a key parameter when deciding on the preferred option.

This report has demonstrated that, at least using the CryoTop tool, these surplus embryos can be successfully revitrified without any apparent detriment. The initial instances of embryo recryopreservation occurred when embryo transfer was cancelled after the embryos had been warmed for both medical and personal reasons, but the practice was later extended as an option for patients with large numbers of cryopreserved embryos. Based upon these results, PIVET moved to use recryopreservation as part of the management strategy for cryopreserved embryos. The increased use of minimal stimulation and blastocyst culture could minimize the scenarios where recryopreservation is needed. However, one case involving oocyte cryopreservation, included in this report, could routinely involve recryopreservation where uncertainty over fertilization rates regularly generates surplus embryos.

The capacity to revitrify and warm embryos that have already been cryopreserved is indicative that vitrification causes minimal cryoinjury, both physically as minimal blastomere lysis and metabolically by retaining cleavage ability after warming. The ability to revitrify mouse embryos repeatedly has been reported by Sheehan et al. (2006), who demonstrated that the metabolic impact of repeat vitrification was minimal. They argued that the avoidance of ice-crystal formation is a key advantage with this method. There have been other reports of isolated cases of recryopreservation by slow freezing (Baker et al., 1996; Yokota et al., 2001) and revitrification (Kumasako et al., 2009; Takahashi and Araki, 2004). The current series extends these observations to a clinical level where recryopreservation was employed, both in crisis situations and as part of a clinical management strategy.

The ability to survive vitrification with very high rates of survival may be influenced by the cryotool employed. The Cryotop method of Kuwayama et al. (2005a,b) facilitates the removal of virtually all surplus cryopreservation media such that the embryo is covered only by a thin meniscus of media. In this study centre, the survival rate is similar to that observed in the rest of the cryoprogramme. Indeed, the high pregnancy rate in this selected cohort of embryos is as good as is routinely observed, although the number of cases in this report is too small for meaningful comparisons. Further support for the safety of this technique comes from the delivery of 11 healthy live births. These results extend and support other observations of the safety of the vitrification and the revitrification technique (Chain et al., 2008; Murakami et al., 2011; Smith et al., 2005).

The Cryotop method described in this report is an open system in that the embryo is exposed to 'clean' liquid nitrogen and the tool is not enclosed in a sealed container, only a protective sheath. Increasingly, vitrification methods are moving to closed systems and the results for recryopreservation need to be confirmed for each method, although they are unlikely to differ substantially from observations (Kuwayama et al., 2005b). Additionally, the current report extends observations (Kumasako et al., 2009) to include embryos cryopreserved initially by either slow freezing or vitrification (Stehlik et al., 2005). The survival and implantation rate after repeated cryopreservation by vitrification appears to be independent of the original method for freezing.

The ability of once-cryopreserved embryos to survive a second round of cryopreservation raises questions as to what happens to the embryo during cryopreservation. One publication on late-stage mouse embryogenesis has identified cold-shock proteins that contribute to protection of cells during periods of 'proliferative stress' (Lu et al., 2006). In the present study, while the revitrified embryos demonstrated competence by both surviving the initial cryopreservation procedure and in most cases continuing to divide, while the embryos have demonstrated competence by both surviving the initial cryopreservation procedure and after re-vitrification, the high implantation rate of these embryos in this study suggests that this may be due to either their initial good quality, enhanced viability due to the vitrification or significant accumulation out of

phase development relative to the implantation window during the transfer cycle. This last point is raised because most frozen embryo transfer programmes routinely transfer embryos 1 day earlier relative to the rise in progesterone. Revitrification means that the stage of embryo development may well be considerably out of phase relative to the uterine development at the time of transfer. Their viability appears independent of the initial method of cryopreservation (slow freezing or vitrification) and the embryo stage at cryopreservation. Further studies need to be undertaken to demonstrate that recryopreservation is a safe procedure to employ routinely.

In summary, cryopreserved warmed embryos may be recryopreserved by vitrification with subsequent high rates of survival and implantation potential. This technique may be a useful resource to consider when developing a management strategy for patients with high numbers of embryos in cryostorage or in emergency situations.

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