

Human Fertilisation and Embryology Authority

The Scientific and Clinical Advances Group

Committee:	Scientific and Clinical Advances Group
Meeting Date:	24 th November 2005
Agenda Item:	7
Paper Number:	SCAG(11/05)03
Paper Title:	Vitrification
Author:	Hannah Darby
For Information or Decision?	Decision
Resource Implications:	
Recommendation to the Committee:	<p>Members are asked to:</p> <ul style="list-style-type: none"> • Note the information presented here and consider the summary of studies regarding the clinical use of vitrification; • Consider the clinical use of vitrification and form a view on its safety and efficacy; • Decide whether the Authority should be advised of any concerns regarding this technique.

1. Background

1.1 From our horizon scanning work vitrification was identified, and prioritised, as an issue that will have an impact on assisted reproduction in the near future. Following the questionnaire sent to the Horizon Scanning Expert Panel two members identified vitrification as being an issue which may begin to impinge upon clinical practice in the UK and we are aware that some clinics may already be using the technique. A briefing paper regarding vitrification was then presented to members at the September SCAG meeting and members received a presentation, regarding vitrification, from Maureen Wood.

2. Information about the technique

2.1 Vitrification is a process that produces a glasslike solidification of living cells that completely avoids the formation of potentially damaging ice crystals during cooling and warming. Vitrification can be achieved in human oocytes and embryos using high cooling rates (e.g. direct plunging into liquid nitrogen) in combination with a high concentration of cryoprotectant. A higher concentration of cryoprotectant is required to prevent devitrification during warming, but this increases the chance of toxic and osmotic injury.

2.2 Exposure of eggs and embryos to the concentrated antifreeze solutions must be very precisely timed to minimise damage. In contrast, exposure to freezing solutions for up to 30 minutes at room temperature is considered safe, thus allowing some leeway if any technical problem arises eg whilst embryos are being loaded into straws. Importantly, because of the need to time precisely exposure to the cryoprotectant solutions it is unlikely that eggs or embryos from more than one patient would be removed from the incubator. For freezing it is common practice to prepare samples for several patients simultaneously.

2.3 Vitrification is a quicker process than freezing for small, but not for larger samples of eggs and embryos (10-15 minutes for one or two eggs or embryos vs ~ 2h to freeze samples of any size) and requires less equipment than conventional slow freezing.

2.4 The ultra-small volumes of sample used in some vitrification strategies may increase the risk of accidental (and lethal) warming during storage. The majority of vitrification strategies reported recently bring the eggs or embryos into direct contact with liquid nitrogen to enhance the rate of cooling. However, this increases the risk of contamination with pathogens. As can be understood from the papers, all reported births are from blastocysts or oocytes vitrified by methods that bring them into direct contact with liquid nitrogen.

2.5 To date the 'universal' vitrification protocol remains to be defined and the technique has to be adapted according to the cell type i.e. oocytes compared to blastocysts.

2.6 It is possible that vitrification will lead to better survival rates for oocytes and embryos compared to that for freezing. However, at present there are not enough data to support this. Also, there are not enough data to conclude as to whether cleavage stage or blastocyst stage vitrification is more successful.

2.3 The safety of vitrification has been researched less rigorously than the safety of freezing, although the data available, eg chromosome counts after parthenogenetic activation of vitrified mouse oocytes, suggest that vitrification is no more deleterious than freezing when exposure to the antifreeze is precisely controlled.

3. Literature review

3.1 Vitrification of embryos/blastocysts

3.1.1 There have been approximately 287 live births from vitrified blastocysts reported.

Reference	Number of blastocysts vitrified and warmed	Number of blastocysts which survived	Number of blastocysts transferred	Number of recipients	Number of live birth events	Number of babies
Takahashi et al., (2005)	1129	716	716	413 cycles	108	147
Zech et al (2005)	186	128	116	148 cycles	10 (60 ongoing pregnancies)	11
Hredzach et al (2005)	215	150	103	42 cycles	7	8
Son et al. (2005)	10	2	2	1	1	2
Mukaida et al (2001)	60	38	36	19	1 (4 ongoing pregnancies)	1
Mukaida et al (2003)	725	583	493	180	18 (37 ongoing pregnancies)	23
Reed et al. (2002)	15	15	13	13	1	1
Yokota et al. (2001)	45	36	32	18	1	1
Stehlik et al. (2005)	41	41	41	20	10	14
Cho et al. (2002)	120	101	92	41	11	15

Choi et al. (2000)	93	48	38	20	5	7
Huang et al (2004)	96	74	60	13	4 (1 ongoing pregnancies)	8
Vanderzwalmen et al. (2002)	167	162	68		10	10
Son et al. (2002)	120	101	92	41	11	15
Son et al. (2003)	90	81	69	25	9 (2 ongoing pregnancies)	15
Yokota et al (2000)	2	2	2	1	1 ongoing pregnancy	
Mukaida et al (1998)	52	52	41	18	1	2
El danasouri (2001)	215	106	106	36	2 (9 ongoing pregnancies)	3
Raju (2005)	127	121			4 (9 ongoing pregnancies)	4

3.1.2 Zech et al (2005) found that partially or completely hatched blastocysts can be cryopreserved by a simple vitrification procedure and they obtained pregnancy rates ranging from 21-35% according to the state of the zona pellucida. Twenty four hours after warming survival rates of 82%, 72% and 64% were observed for blastocysts with open zona pellucida, open and intact zona pellucida and intact zona pellucida respectively.

3.1.3 Hredzak et al. (2005) used a modified method of vitrification to freeze 215 human embryos after IVF-ICSI, obtained in 42 cycles. The method consisted of a series of solutions being used with increasing cryoprotectant concentrations. 69.8% of embryos survived thawing and 48% were capable of further development. The mean number of embryos transferred were 2.9 ± 1.4 . The percentage of clinical pregnancies per thawing cycle and per transfer was 19% and 27.6% respectively. Seven patients delivered 8 children.

3.1.4 Son et al. (2005) reported the delivery of healthy twins from repeat vitrification and thawing of blastocysts derived from in vitro matured oocytes.

3.1.5 Mukaida et al (2001) carried out a clinical trial of vitrification of human blastocysts. A total of 60 vitrified blastocysts from 21 patients were warmed, and the survival rate at 2 hours after warming was 63%. Six clinical pregnancies were achieved after 19 transfers. One healthy baby was born, four pregnancies were ongoing and one ended in miscarriage. In 2003 the same group reported the vitrification of 725 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 significantly higher than that of day 6 blastocysts (55%), but implantation rates and pregnancy rates were not statistically significantly different.

3.1.6 Reed et al. (2002) reported that thirteen couples elected to have blastocysts cryopreserved via vitrification (cryoloop method). Of these thirteen, four couples returned for cryopreserved embryo transfer. Fifteen blastocysts were thawed, 100% were recovered and deemed viable. Thirteen were replaced and one transfer resulted in the birth of a healthy baby.

3.1.7 Yokota et al (2001) carried out a retrospective study of blastocyst vitrification. Of 45 vitrified blastocysts, 36 survived (80%). The implantation rate was 21.9% (7 of 32) and the pregnancy rate was 33.3% (6 of 18). One of the pregnancies resulted in the delivery of a healthy baby.

3.1.8 Kumasako et al (2005) reported a successful birth after the transfer of postthawed human zygotes that were vitrified using a conventional straw. After the vitrification and thawing of four zygotes, two embryos were transferred, resulting in the birth of a healthy boy.

3.2 Studies comparing vitrification with slow freezing and fresh cycles

3.2.1 These studies are retrospective and it is unclear how embryos were assigned to treatment.

3.2.2 A recent study by Takahashi et al., (2005) concluded that the vitrification of blastocysts using cryoloop is effective and safe in clinical use. This study evaluated all the vitrified-warmed blastocyst transfers carried out in a Tokyo clinic between April 2000 and June 2003. During this period the clinic performed 435 cycles of vitrified-warmed blastocyst transfers resulting in the birth of 147 babies. The survival rate of vitrified blastocysts was 85.7%. No statistical difference was seen for gestational age, birth weight, number of preterm deliveries, number of twins and triplets between the group which received fresh embryos and that which received vitrified embryos. However, the male ratio was significantly higher for fresh embryo transfers.

Cycles	Fresh	Vitrification
No. of cycles transferred	602	413
No. of embryos transferred	1,252	716
Number of deliveries	153	108
Number of live born infants	205	147

3.2.3 Stehlik et al (2005) carried out a retrospective study to compare a slow freezing protocol to a vitrification protocol for cryopreservation of day 5 and day 6 human blastocysts. To demonstrate this the survival, implantation rate and pregnancy rates were compared after thawing, assessment and embryo transfer of 86 consecutive day 5 and day 6 thawed blastocyst transfer cycles. 59 out of 71 slow frozen blastocysts survived the thawing process. An average of 2.5 of these blastocyst were replaced per embryo transfer, resulting in a pregnancy rate of 16.7%. All of the 41 vitrified blastocysts which were thawed survived the process. An average of 2 vitrified blastocysts were replaced per embryo transfer, resulting in a pregnancy rate of 50%. So survival, pregnancy and implantation rates of day 5 blastocysts were found to be significantly higher for vitrified blastocysts compared to slow-frozen blastocysts.

	Slow freezing	Vitrification
Number of transfers	51	35
Survival rate	86.3%	100%
Number of pregnancies	9	15
Number of implantations	9	21

3.2.4 Raju et al (2005) reported a prospective study comparing the outcome for vitrified embryos compared to slow frozen embryos. The excess embryos after embryo transfer of 164 patients were randomly divided and cryopreserved vitrification or slow freezing. There was no significant difference between mean age and number of embryos transferred in the two groups. The post thaw survival rate of the embryos in the vitrification group was significantly higher than for embryos in the slow freezing group.

	Slow freezing	Vitrification
Number of embryos cryopreserved	420	436

Number of embryos thawed	120	127
Number of embryos survived	72	121
Number of transfer cycles	23	40
Number of pregnancies	4	14
Number of deliveries	2	4
Number of ongoing pregnancies	1	9

3.3 Studies for vitrification of oocytes

3.3.1 Kuwayama et al (2005) compared a number of vitrification methods. They found that the Cryotop method (vitrification in <math><0.1\mu\text{l}</math> medium droplet on the surface of a specially constructed fine polypropylene strip attached to a plastic handle) yielded the best results. Out of 64 vitrified oocytes, 58 (91%) exhibited normal morphology after warming. After intracytoplasmic sperm injection, 52 became fertilised, and 32 (50%) developed to the blastocyst stage *in vitro*. Analysis by fluorescence in-situ hybridisation of five blastocysts showed that all were normal diploid embryos. Twenty-nine embryo transfers resulted in 12 pregnancies (41% pregnancy rate), seven healthy babies and three ongoing pregnancies. It was suggested that reducing the CPA concentration of vitrification solutions may be one reason for the improved survival after the vitrification procedure used in this study.

3.3.2 Yoon et al. (2003) assessed the usefulness of the vitrification method in clinical practice. Surplus oocytes from 34 IVF-embryo transfer patients were vitrified for the next cycle. The overall morphological survival and fertilisation rates of the vitrified/thawed oocytes were 68.6% and 71.7% respectively. All 6 pregnancies resulted in the delivery of healthy babies (1 twin and 5 singletons).

3.3.3 Kyono et al (2005) reported the birth of a healthy male baby following the transfer of a single blastocyst derived from vitrified mature human oocyte and donor sperm.

3.3.4 Yoon et al (2000) reports the vitrification of 90 oocytes following unsuccessful fresh embryo transfer for 7 patients. The proportion of these oocytes with a first polar body and normal cytoplasm, the proportion fertilised by ICSI and the proportion that developed into the pronuclear stage were not significantly decreased when compared with those in fresh cycles. A total of 32

cleaving embryos from vitrified oocytes were transferred to 7 patients, and three patients achieved a singleton pregnancy.

3.3.5 Kuleshova et al (1999) reported a case study of four patients who agreed to have their surplus oocytes vitrified with the intention of reducing the number of surplus zygotes and embryos that need to be frozen. A total of 17 oocytes from the four patients were vitrified. Two to seven of the mature oocytes from the four patients were randomly allocated to the vitrification study. Of the 17 oocytes 11 survived intact and were injected with spermatozoa. Three of these embryos were transferred to three patients resulting in one live birth.

4. Timescale

4.1 Vitrification procedures are used for cryopreservation of ovine, equine, murine, rabbit, bovine and porcine oocytes and embryos at all stages of embryonic development. Vitrification of human cleavage stage embryos and blastocysts has resulted in the birth of healthy babies.

4.2 Although techniques for oocyte cryopreservation are not so advanced reports in animals have demonstrated that vitrification can be used for storing at both the germinal vesicle stage and the metaphase II stage. A small number of livebirths have been reported after the transfer of embryos derived from vitrified oocytes.

4.3 Vitrification is already being used in treatment in other countries and possibly by some clinics in this country. Also, the HFEA has licensed research projects on the development of vitrification methods for human embryos and oocytes.

4.4 Equipment for vitrification is available commercially e.g. the cryoloop and cryovial (Hampton Research, Laguna Niguel CA), Vitrolife (Irvine Scientific), Medicult medium, Hunter scientific products, cryoprotectant solution (Pharmacia Biotech, Sweden), Dulbecco's, cryotop device (Kitazato, Fuji-shi, Japan), electron microscope grids used for vitrification (Pelco International, CA, USA).

4.5 The vitrification method marketed by Medicult, which involves using the McGill cryoleaf places the embryos in direct contact with liquid nitrogen. Medicult recommend the use of a sterile container (e.g. pyrex beaker, which is a dangerous practice that would be against health and safety regulations in the UK and sterile liquid nitrogen). It is questionable whether liquid nitrogen can be sterilised effectively. At least one kit (Vitrolife) prevents direct contact to liquid nitrogen by sealing the straws.

5. Regulatory framework

5.1 Currently, if a centre wishes to carry out vitrification they would not have to apply for a variation to their licence. Also, there is no specific requirement for clinics to inform the HFEA that they are adopting practices such as vitrification in the course of treatment.

5.2 It is expected that the HFEA will be informed when a clinic introduces a new practice. This requirement is set out in Appendix H (standard licence conditions applicable to all licences) to the Code of Practice. It states that:

“Where the centre proposes to introduce new activities or treatment services not specified in the licence, these may not be commenced until notification has been given to the Authority and where the Authority considers it necessary an application has been made to the Authority for a licence relating to the new activities and such a licence has been granted.”

However, vitrification cannot be classed as a ‘new activity or treatment service’ as it is simply a variation of storage procedures.

6. Previous discussion

6.1 At the September 2005 SCAG meeting it was decided that members should decide whether the HFEA needs to issue guidance to clinics about the clinical use of vitrification. It was also suggested that using a large number of human oocytes, necessary to obtain concrete evidence regarding the safety of the technique for human oocytes, may be considered unethical.

6.2 It was noted that centres would not be required to apply for a licence to carry out vitrification. However, there is a requirement for clinics cryopreserving eggs/embryos to submit data. Members suggested that if clinics carry out vitrification the HFEA could request that they submit data regularly.

7. Conclusions/recommendations

7.1 The Executive recommend that SCAG:

- Note the information presented here and consider the summary of studies regarding the clinical use of vitrification;
- Consider the clinical use of vitrification and form a view on its safety and efficacy;
- Decide whether the Authority should be advised of any concerns regarding this technique.

8. References

- Cho et al. An improved protocol for dilution of cryoprotectants from vitrified human blastocysts. *Human Reproduction* 2002; 17: 2419-2422.
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