

OBJECTIVE: Storage of cryopreserved oocytes in liquid nitrogen is very demanding in terms of maintenance, storage space, equipment and costs. Such demands are predicted to increase as more women are resorting to oocyte cryopreservation to preserve their future fertility options. We therefore sought an alternative method for gamete preservation: lyophilization and dry storage.

DESIGN: Experimental Research.

MATERIALS AND METHODS: Three experiments in parallel compared various cooling methods on the recovery and survival after freeze/dry of in vitro-matured MII bovine oocytes (n=68) inclusive of cumulus cells. Ten oocytes were cryopreserved with slow freezing (using MTG 1314 device) at a cooling rate of 4°C/minute (group A); 24 oocytes with rapid freezing (using MTG 516 device), at a cooling rate of 150°C/minute (group B); and 34 with vitrification using minimum drop size in IMT-4 solution (mix of cryoprotectants and trehalose) at a cooling rate >20,000°C/minute. All oocytes were prepared for cryopreservation using IMT-4 solution. The lyophilization process was carried out with the VirTis wizard for 24 hours with shelf temperature of -55°C and vacuum 10 mTorr. The rehydration process took place at room temperature using equilibrated TCM199 supplemented with 0.5M trehalose and 10% BSA. Oocyte survival was assessed with the live/dead staining (SYTO/PI). Control oocytes were stained fresh.

RESULTS: For group A, 70% of the oocytes were recovered after rehydration but only 1/7 stained as viable (14%); for group B, 71% were recovered and 10/17 stained as viable (59%). For group C, 88% were recovered and 23/30 stained as viable (77%) (P<0.05). The granulosa cells viability was the highest (95%) within the vitrification group.

CONCLUSION: 1) Lyophilization of oocytes is a groundbreaking innovation for gamete bio-banking; 2) Vitrification is confirmed as an essential method not only for preservation in liquid nitrogen but also for a dry state.

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REPRODUCTIVE TECHNOLOGY: THE EFFECTS OF CONTEXT ON ATTITUDES. B. A. Dooley J. D. Hans. Family Sciences, University of Kentucky, Lexington, KY.

OBJECTIVE: To examine the effect of contextual circumstances on attitudes towards reproductive technology.

DESIGN: A multiple-segment factorial vignette was designed to explore the effects that contextual circumstances (i.e. sex of the individual seeking treatment, relationship status, age, source of gametes, and sexual orientation) have on attitudes toward the procurement of assisted reproductive technology services. A probability sample of ROUGHLY 400 Kentucky households was obtained using random-digit dialing that gave every household in the state of Kentucky an equal probability of being contacted.

MATERIALS AND METHODS: Descriptive statistics, three logistic regression models, three ordinal regression models, and over 1,000 open-ended rationales provided by participants for their closed-ended responses, will be summarized.

RESULTS: Preliminary analyses indicate that procurement of assisted reproductive technology services was viewed as more appropriate when sought by older than younger individuals, by single women than couples and by couples than single men, and by lesbians than by gay males. Also, female respondents generally viewed the use of assisted reproductive technology as more acceptable than did male respondents.

CONCLUSION: Attitudes toward reproductive technology vary according to context and it is important to understand the nuances of these differences and the reasons for them to inform medical decision making and policy development.

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CUMULATIVE PREGNANCY AND BIRTH RATES WITH THE USE OF FRESH AND VITRIFIED/THAWED OOCYTES. R. E. Nakamo,^a C. G. Almodin,^b V. C. M. Camara,^b L. S. Fujihara,^a C. M. O. Filho,^d A. P. Ceschin,^c ^aHuman Reproduction, Ferticlin, São Paulo, SP, Brazil; ^bHuman Reproduction, Materbaby Reprodução Humana, Maringá, PR, Brazil; ^cHuman Reproduction, Felicidade Instituto de Fertilidade, Curitiba, PR, Brazil; ^dHuman Reproduction, Nucleo Santista de Reprodução Humana, Santos, SP, Brazil.

OBJECTIVE: Recent reports in the literature have demonstrated that pregnancy rates obtained from vitrified/thawed oocytes are comparable to those

using fresh cycles, suggesting that oocyte vitrification may become an alternative to embryo freezing. The objective of this study was to determine the benefit of oocyte vitrification in a group of typical infertile couples who underwent IVF treatment and had their surplus oocytes vitrified.

DESIGN: Prospective study in which cumulative pregnancy and birth rates obtained from the fertilization of fresh and vitrified/thawed oocytes from the same cohort were calculated.

MATERIALS AND METHODS: Surplus oocytes obtained from a total of 81 patients between May 2009 and November 2010 were vitrified and stored. Those patients that did not carry their gestation to term after the first IVF attempt (fresh cycle), had their stored oocytes thawed for a second attempt and, in some cases, a third attempt (vitrified/thawed cycles).

RESULTS: A total of 992 oocytes in MII were collected after ovarian stimulation, from which 515 were vitrified. After the first IVF attempt using fresh oocytes, 40 patients got pregnant (49.4%), from which 23 carried their gestation to term (28.4%). Unsuccessful patients had their stored oocytes thawed (237), resulting in 67 new embryo transfer cycles, with 24 patients getting pregnant (35.8%), among which 18 had babies (26.9%), producing a cumulative pregnancy rate of 79.0% and a birth rate of 50.6%, respectively.

CONCLUSION: The results obtained in this study demonstrated that oocyte vitrification can be beneficial in the treatment of a typical infertile population. It may avoid the legal restrictions concerning the number of embryos that can be transferred, as well as the ethical and religious problems involved with their cryopreservation. Furthermore, it can also be more cost effective to the patient, who does not require new stimulation procedures.

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LOWER INTRACELLULAR CONCENTRATION OF CRYOPROTECTANT (ICCP) AFTER VITRIFICATION (VIT) THAN SLOW FREEZING (SF) DESPITE EXPOSURE TO HIGHER CONCENTRATION OF CRYOPROTECTANT SOLUTIONS (CPS). P. Vanderzwalmen,^a L. Grobet,^b N. H. Zech,^a F. Puissant,^c F. Ectors,^b ^aIVF Centers Prof. Zech, Bregenz, Austria; ^bGIGA Research, University of Liege, Liege, Belgium; ^cCentre Hospitalier Inter Régional Cavell Chirec, Bruxelles, Belgium.

OBJECTIVE: Seeing that VIT is on the way to replace SF, a hot debate is set on about the exposure of oocytes or embryos to cryoprotectant solutions (CPs) that exceed 3 to 4 those in SF, bringing concerns about their toxicity.

DESIGN: The aim of our study is to bring some insights about the question how high the ICCP are after VIT or SF. Mouse zygotes were cryopreserved either by VIT or SF. The ICCP was evaluated by analyzing the rate of intact 2PN in relation with the rate of warming and to their resistance to the osmotic stress during dilution step in different sucrose solutions (SUS).

MATERIALS AND METHODS: SF (0.3°C/min to -35°C and drop in LN2) or VIT was applied on FVB/N zygotes. Before SF, the 1-cell embryos were equilibrated for 10 - 15 min. in a solution of 1.5M CP. For VIT, the 2PN were exposed to CPs of 4.2 and 6.4 M for 8 and 1 min. before being plunged in LN2.

Experiment 1:

The higher the ICCP, less is the probability of recrystallization and cell-lysis when reduced warming rate is applied.

Fast (plunge in water or solution at 37°C) and slow (keep in air) warming procedures were applied after SF and VIT.

Experiment 2:

The higher the ICCP, the higher the SUS is necessary to minimize over swelling due to fast entrance of H2O. Insufficient SUS will result in the lysis of the 2PN. After fast warming, the 2PN are diluted in SUS ranging from 0M to 1M.

For each experiment the rate of intact 2PN was recorded. Three assays of ten 2PN each were performed in each group.

RESULTS:

TABLE 1.

% of intact 2PN after SF or VIT in relation with the warming rate		
Warming	SF	VIT
In air	80*	10*
37°C	77	100
% of intact 2PN after SF or VIT in relation with the SUS concentration		
Sucrose (M)	SF	VIT
0	7 *	37 *
0.25	43	77
0.5	70	97

* P<0.01.