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A prospective comparison of outcome following cryopreservation using vitrification *vs.* a modified slow-freeze protocol of 2 pronuclear (2PN) and day 3 multi-cell embryos

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Summary

Purpose: To compare the efficacy of vitrification of 2 pronuclear and day 3 cleavage stage embryo *vs.* a modified slow freeze protocol that historically has achieved good survival and pregnancy rates at these stages. *Materials and Methods:* Embryos were randomly assigned by day to freezing at the 2 proncular stage or day 3 cleavage stage embryos by either vitrification or a modified slow freeze protocol. Comparisons were made for survival rate, cleaveage rate, and pregnancy rate. *Results:* The results were comparable with a slight edge to vitrification. Only the implantation rates of day 3 cleavage staged embryos (75% *vs.* 30.4%) showed a significant difference. *Conclusions:* Vitrification seems to be equally or possibly slightly superior to freezing embryos at the 2 pronuclear or day 3 cleavage stage *vs.* a modified slow freeze protocol that had been previously found to be superior to the slow freeze method of LaSalle-Testart.

Key words: Vitrification; Modified slow freeze; 2 pronuclear embryos; Day 3 cleavage stage embryos; Frozen embryo transfer.

Introduction

Many in vitro fertilization-embryo transfer (IVF-ET) centers did not fare well with the LaSalle-Testart slow-cool embryo cryopreservation protocol. Many of these centers are enjoying much better survival and pregnancy rates using the rapid freeze technique known as vitrification.

For many years the present IVF center used a modified simplified slow cool technique with a one-step removal of the cryoprotectant 1,5-propanediol combined with assisted hatching and enjoyed a pregnancy rate following frozen ET comparable to the pregnancy rate following fresh ET [1-4]. Many centers seem to prefer to freeze blastocysts by vitrification. There does not seem to be many studies on freezing of 2 pronuclear embryos with this technique.

The objective of the present study was to compare the efficacy of vitrification *vs.* modified slow-cool technology on embryo survival of 2PN and day 3 cleavage stage embryos and pregnancy rates.

Materials and Methods

A prospective randomized controlled study was performed. Embryo cryopreservation by vitrification was performed on three fixed days per week and slow freeze on four days per week. Brief summary of vitrification technique: Irvine media, six to ten minutes in equilibration solution, 90 seconds in vitrification solution at room temperature, Next loaded into high security vitrification straw in < one µl of medium, sealed, and plunged into liquid nitrogen.

Brief description of modified slow-freeze: Equilibrate in modified human tubal fluid +10% serum protein substitute for ten minutes then placed in 1.5M propanediol for up to 20 minutes, then loaded in straw and seeded, then cooled from -6°C to -40°C at ramp rate of 0.4°C/minute in alcohol bath freezer (BioCool), then plunged in liquid nitrogen. Comparisons were made of survival and cleavage rates and pregnancy rates according to method of freezing in women aged ≤ 39.9 years. The comparisons were also stratified according to the stage of freezing: 2 pronuclear *vs.* day 3 cleavage stage. Statistical analysis was performed by either chi-square analysis or Fisher's exact test when appropriate.

Results

The survival and cleavage rates of 2 pronuclear embryos and pregnancy rates following ET according to the method of freezing in women aged ≤ 39.9 years are seen in Table 1.

Table 1. — *Survival and cleavage rates and pregnancy rates of 2 pronuclear embryos according to the method of freezing.*

	Survival rate	Cleavage rate (more than 1 cell)	Clinical pregnancy rate	Implantation rate
Vitrification	97.2% (35/36)	94.3% (33/35)	75% (6/8)	50% (4/8)
Slow freeze	98.8% (92/96)	94.6% (87/92)	53.3% (8/15)	30% (10/30)

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Table 2. — *Survival and cleavage rates and pregnancy rates of day 3 cleavage stage only.*

	Survival rate (≥ 50% blastomeres)	Cleavage rate (more than 1 cell)	Clinical pregnancy rate	Implantation rate
Vitrification	91.5% (65/71)	90.7% (59/65)	47.1% (8/17)	75% (12/16)
Slow freeze	88.8% (40/45)	95% (38/40)	46.2% (6/13)	30.4% (7/23)

The only comparison showing a significant difference was the implantation rate of day 3 cleavage stage embryos favoring vitrification ($p < 0.05$, Fisher's exact test). The survival and cleavage rates of day 3 embryos and pregnancy rates following ET according to the method of freezing in women aged ≤ 39 years are seen in Table 2.

Discussion

Vitrification appears to be at least equally effective and possibly slightly superior to the modified slow freeze protocol even when using 2 pronuclear and day 3 cleavage stage embryos. The only advantage of the modified slow cool technique is that it is less expensive. Looking at it another way, those few centers using the modified slow cool technique and having success, do not need to switch to vitrification as so many other centers have done who had used the LaSalle-Testart technique to improve pregnancy and survival rates. They seem to be comparable with perhaps just a slight edge to vitrification.

References

[1] Baker A.F., Check J.H., Hourani C.L.: "Survival and pregnancy rates of pronuclear stage human embryos cryopreserved and thawed using a single step addition and removal of cryoprotectants". *Hum. Reprod. Update*, 1996, *2*, 271.

[2] Check J.H., Hoover L., Nazari A., O'Shaughnessy A., Summers D.: "The effect of assisted hatching on pregnancy rates after frozen embryo transfer". *Fertil. Steril.*, 1996, *65*, 254.

[3] Check J.H., Choe J.K., Nazari A., Fox F., Swenson K.: "Fresh embryo transfer is more effective than frozen ET for donor oocyte recipients but not for donors". *Hum. Reprod.*, 2001, *16*, 1403.

[4] Check J.H., Katsoff B., Wilson C., Choe J.K., Brasile D.: "Pregnancy outcome following fresh vs. frozen embryo transfer into gestational carriers using a simplified slow freeze protocol". *Clin. Exp. Obstet. Gynecol.*, 2012, *39*, 23.

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