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Editors

VICTOR GOMEL, M.D.  
PETER C.K. LEUNG, Ph.D.



MONDUZZI EDITORE

*INTERNATIONAL PROCEEDINGS DIVISION*

# Analysis of post-thaw survival and pregnancy rates of multi-cell embryos cryopreserved and thawed using a simplified protocol

D. SUMMERS, A. BAKER, J.H. CHECK  
and J. CHOE

*The University of Medicine and Dentistry of New Jersey  
Robert Wood Johnson Medical School at Camden  
Cooper Hospital/University Medical Center  
Department of Obstetrics and Gynecology  
Division of Reproductive Endocrinology and  
Infertility, Camden, NJ (USA)*

## SUMMARY

873 embryos cryopreserved at the multi-cell stage using a one-step method were thawed for use in 251 embryo transfer (ETs). 70% of the embryos survived (at least 50% of their cells were intact), 42.5% of the embryos were completely intact. There were 31 (12.3%) clinical pregnancies and an implantation rate of 5.6%. Although these data demonstrate that the simplified cryopreservation-thawing protocol provided good survival and PRs, these rates were lower than those reported for cryopreserved human zygotes possibly because multi-cell embryos were frequently "leftovers" with the embryos with best morphology having been transferred fresh.

## INTRODUCTION

The demand for cryopreservation of human embryos is constantly increasing. Besides its use for supernumerary embryos, cryopreservation of all embryos is recommended in many situations where the clinician feels that transfer should be deferred until a more receptive uterine environment can be achieved. For example, it is now used as a preventive measure for patients at risk of ovarian hyperstimulation syndrome (OHSS) (Rizk et al, 1991). It is also used in cases where inadequate endometrial development is observed (i.e., either a thin endometrium or a homogeneous hyperechogenic echo pattern) (Check et al, 1991). Thus, the cryopreservation and thawing protocol is crucial to the success of the program.

At our center, we modified a simplified cryopreservation thawing method used for bovine morula and adapted it for humans. We have reported a 87.4% survival rate upon thawing of human zygotes and a 21.4% pregnancy rate (PR) following embryo transfer (ET) (Baker et al, 1997). The objective of this study was to evaluate the efficacy of this method on multi-cell embryos.

## MATERIALS AND METHODS

Multi-cell embryos with 2-8 cells were cryopreserved using a simplified one-step method (Baker et al, 1997). In this procedure, the embryos were equilibrated in 1.5 M 1,2 propanediol (PrOH) and frozen in 0.25 mL straws in an alcohol bath controlled rate freezer. The straws were preloaded with 0.12mL of 1 M sucrose followed by 1cm air column and then a column of PrOH containing an embryo. Seeding was performed at -6°C. The straws were cooled at -0.4°C/min down to -40°C and then stored in liquid nitrogen.

873 embryos were thawed for use in 251 frozen ETs. The PrOH was diluted out in one step after vigorously shaking the straw to mix the sucrose with the embryo (Baker et al, 1997). A multi-cell embryo was said to survive if at least 50% of its cells remained intact after the thaw.

Stimulation protocols for frozen ET included natural cycles and hormone replacement therapy with and without down regulation. Embryos with 25-50% of cells intact was transferred along with all embryos that survived at patient's request. Clinical pregnancy was defined as sonographic evidence of gestational sac in the uterus. Implantation rates were computed as number of gestational sacs per embryo transferred.

## RESULTS AND CONCLUSIONS

Seventy percent of the 873 embryos thawed survived with at least 50% of their cells intact. When classified by the cell stage, the highest survival rate was observed in 2 cell embryos (80.9%, 140/173). For 3-8 cell embryos, the survival rates ranged from 60.3% to 70.9%, tab. 1. 371 embryos (42.5%) were completely intact after thawing, i.e., all cells were intact. Again, two cell embryos had the highest percentage of embryos in which all cells remained intact following thaw (tab. 1).

There were 31 clinical pregnancies achieved following 251 ETs for a PR of 12.3% and an implantation rate of 5.6%. An average of 2.9 embryos were transferred per cycle. Classifying the cycles by number of embryos transferred, the PRs were 4.5% if only 1 or 2 embryos were transferred;

Table 1 - Survival of embryos cryopreserved at the multi-cell stage

Cell stage at time of freeze	# of embryos thawed	# of embryos surviving*	# of embryos completely intact
2	173	140 (80.9%)	109 (63.0%)
3	123	81 (65.9%)	56 (45.5%)
4	374	265 (70.9%)	152 (40.6%)
5	116	70 (60.3%)	31 (26.7%)
6	53	32 (60.4%)	14 (26.9%)
7	16	11 (68.8%)	5 (31.1%)
8	18	12 (66.7%)	4 (22.2%)
Total	873	611 (70.0%)	371 (42.5%)

\*Survival defined as at least 50% of cells intact after thaw

16.5% if 3 embryos were transferred and 17.2% if 4 embryos were transferred.

Although these data demonstrate that the simplified cryopreservation-thawing protocol provides good survival and PRs, these rates were not as high as those reported for human zygotes frozen at the pronuclear stage. These lower rates may be attributable to the fact that fewer embryos were transferred per cycle than in our study on human zygotes and also to the fact that multi-cell embryos were frequently "leftovers" with the embryos with best morphology having been transferred fresh.

#### REFERENCES

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