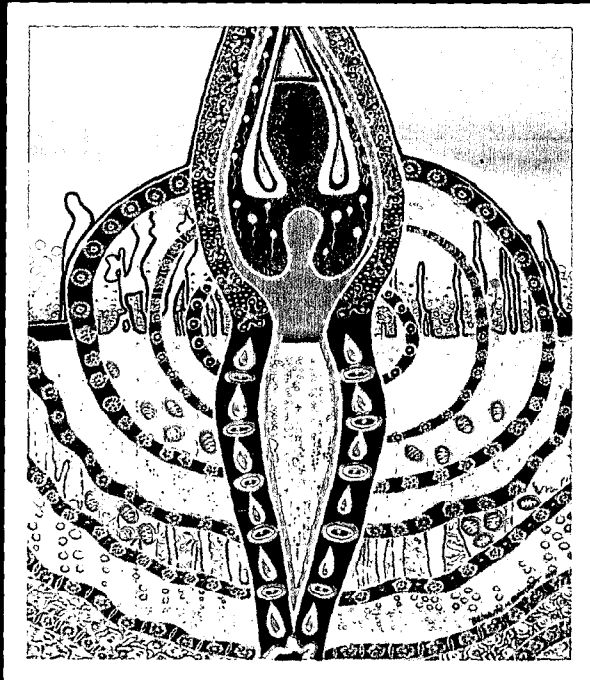


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A Comparison of Embryo Survival Following Thawing of Embryos Using a Simplified Freeze/Thaw Protocol by Age of Patient and Cell Stage at Time of Cryopreservation

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Summary

A retrospective study was performed to compare the survival rate of cryopreserved embryos by the age of the female. It was hypothesized that the lower pregnancy rates obtained by women ≥ 40 following frozen ET could be due to the inability of embryos from older patients to withstand the rigors of the cryopreservation/thaw process even when a simplified freeze/thaw method was used. The survival rates of embryos thawed for use in 1624 frozen ET cycles were computed by stage at which the embryo was frozen and the age of the patients. The 4 age groups were ≤ 35 , 36-39, 40-42, and ≥ 43 years. There were no differences in the survival rate of embryos frozen at the 2 pronuclear stage by age (95.0%, 94.6%, 96.7%, and 94.4%, respectively); nor in the survival rate of embryos frozen at the multi-cell stage (82.1%, 79.2%, 83.6%, and 79.5%, respectively). These data do not support the hypothesis that embryos from older women have less ability to withstand the rigors of cryopreservation.

Introduction

Reports from the FIVNAT registry (1) and the SART registry (2) both report lower pregnancy rates (PRs) in frozen embryo transfer (ET) cycles in women ≥ 40 years old. The question arises as to why women who were successfully stimulated and had supernumerary embryos fail to conceive. Is it a function of oocyte quality, uterine receptivity or the inability of the older embryo to withstand the rigors of the cryopreservation/thaw process?

A report by Munne et al (3) has suggested that older patients have problems related to oocyte quality especially with problems during meiosis, e.g., non-disjunction of chromosomes, resulting in embryos with a higher rate of aneuploidy. Older eggs also have defective mitochondria. Because of these defects, embryos from older patients have lower implantation rates. The retrospective study presented here aimed to determine if these oocyte defects in the aged patient is also reflected in the inability of the embryos to survive the process of cryopreservation and thawing.

Materials and Methods

A retrospective review of all frozen ET cycles performed between November, 1991 and August 1, 1998 was performed. Cycles involving donor oocytes, donor embryos or embryos fertilized through ICSI were excluded. A total of 1624 frozen ET cycles were included. Cycles were stratified into 4 groups based on the age of the woman at time of oocyte retrieval and cryopreservation. The age groups were: ≤ 35 years, 36-39 years, 40-42 years, and ≥ 43 years.

Embryos were cryopreserved and thawed using a simplification of the LaSalle/Testard technique as described previously (4). This method requires only a one-step removal of cryoprotectant. A 2 pronuclear embryo was said to survive the thaw if it appeared intact and cleaved to at least the 2 cell stage after an 18 to 24 hour incubation period. Multi-cell embryos were considered to have survived if at least 50% of cells appeared intact after thawing.

Chi-square analysis was used to compare the survival rates by age group. A p value of .05 was used.

Results

The survival rates of 2 pronuclear stage embryos ranged from 94.4% to 96.7% in the four age groups (Table 1). There were no significant differences in the survival rates by age of the patients at retrieval ($p=NS$). The survival rates of multi-cell embryos ranged from 79.5% to 83.6%. There were no significant differences in survival rates of multi-cell embryos by age ($p=NS$).

Table 1 - Comparison of survival rates by age*

Age at Retrieval				
Embryo stage at cryopreservation	≤35 years (n=1098)	36-39 years (n=372)	40-42 (n=111)	≥43 years (n=43)
Pronuclear stage				
# thawed	3237	1067	305	90
# survived	3076 (95.0%)	1010 (94.6%)	295 (96.7%)	85 (94.4%)
Multi-cell stage				
# thawed	1236	482	104	39
# survived	1015 (82.1%)	382 (79.2%)	87 (83.6%)	31 (79.5%)

*p=NS

Conclusions

These data do not demonstrate any relationship between survival rates of frozen/thawed embryos and age. Irrespective of age, the survival rate of pronuclear embryos was greater than 90%. The survival rate of multi-cell embryos was slightly lower than those for pronuclear embryos, but again there was no difference by age of the woman at time of oocyte retrieval.

The hypothesis that older embryos cannot withstand the rigors of cryopreservation was not supported by these data. If older women can be successfully stimulated and oocytes are retrieved and fertilized, the clinician can be assured that the resultant embryos have the same chance of surviving the freeze/thaw process as the embryos from younger patients. Lower live delivery rates in older patients following frozen ET are probably related to a high rate of aneuploidy, but apparently embryos with chromosomal or mitochondrial defects are equally as likely to survive embryo cryopreservation/thawing at least when this simplified freezing protocol is used (1,2,5).

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