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ASSISTED REPRODUCTION

Viability of frozen-thawed human embryos with one–two blastomeres lysis

Xiaoying Zheng · Ping Liu · Guian Chen · Jie Qiao · Yuqi Wu · Ming Fan

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Abstract

Objective To assess the impact of one–two blastomeres lysis on the viability of thawed day 3 human embryos.

Methods A retrospective analysis was performed on 248 frozen-thawed embryo replacement cycles in which all embryos were frozen at day 3 at the seven–eight cell stage with ≤10% fragmentation.

Results Outcomes of transfer cycles with fully intact embryos (intact group) were compared with those in which all transferred embryos have lost one–two blastomeres (damage group). Comparable pregnancy rates (38.46% vs 38.64%), birth rates (34.62% vs 36.36%) and implantation rates (26.31% vs 26.25%) were obtained in intact and damage groups. These results were also not significantly different from mixed transfer cycles in which one intact embryo and one damaged embryo were transferred together.

Conclusion The developmental potential of partially damaged cryopreserved human embryos with less than 25% cells loss is comparable to that of fully intact embryos. Presence of one–two lysed blastomeres in the thawed day 3 embryo does not appear to have a negative influence on the further development of the sibling intact cells.

Keywords Blastomere loss · Embryo cryopreservation · Implantation

Capsule Presence of one–two lysed blastomeres in the thawed day 3 embryo does not appear to have a negative influence on the further development of the sibling intact cells.

X. Zheng · P. Liu (✉) · G. Chen · J. Qiao · Y. Wu · M. Fan
Department of Ob & Gyn, The Third Hospital, Peking University, Beijing 100083, China
e-mail: licuping@126.com

Introduction

Since the first report on human pregnancy using a frozen embryo [1], embryo cryopreservation has become a widespread reliable routine procedure in assisted reproduction treatment. An efficient embryo cryopreservation method offers the advantage of increasing the cumulative pregnancy rate for a single ovarian stimulation cycle while minimizing the risk of multiple pregnancies. In cases where ovarian hyperstimulation may be anticipated or where embryo transfer may be considered inadvisable for some reasons, including uterine bleeding and/or intracavitary fluid, embryo cryopreservation offers the option of freezing all embryos for thawing and transfer in subsequent, more favorable cycles.

It is well known that only some cryopreserved embryos remain fully intact after thawing even if only the embryos with the best morphological appearance have been frozen. Other embryos lose one or more blastomeres after thawing and are thus referred to as partially damaged. The studies that have been reported so far have provided conflicting results with regard to the developmental potential of partially damaged frozen and thawed embryos. Some scientists have reported that damaged embryos had the same capacity to produce pregnancies as fully intact embryos [2], while others have reported a deleterious effect of embryo damage on their implantation potential [3–6]. The reason for lower viability remains controversial. A possible toxic effect from damaged blastomeres on the other blastomeres has been proposed as a mechanism. The purpose of this retrospective study was to assess the impact of one–two blastomeres loss on the outcome of frozen embryo replacement cycles in which only one embryo or only embryos with equivalent characteristics were transferred.

Materials and methods

Embryos

The analysis was confined to non-donor frozen embryo transfer (FET) cycles involving embryos cryopreserved on postinsemination day 3; cycles using embryos frozen at earlier or later cleavage stages were excluded from the analysis. The embryos included in this study were transferred in procedures from January 2003 to December 2005 in our hospital. Uniform methodology was employed in our clinics over the period of the study. Ovarian stimulation in the oocyte retrieval cycle had been carried out using a down-regulation protocol combining the gonadotrophin-releasing hormone agonist with recombinant human follicle-stimulating hormone. Transvaginal ultrasound-guided oocyte retrieval was performed 34–36 h following human chorionic gonadotrophin (HCG) injection. Oocytes were inseminated either by conventional in vitro fertilization (IVF) or by intracytoplasmic sperm injection (ICSI) according to routine protocols. Fertilization was considered normal when two pronuclei were present between 16 and 18 h after insemination. Only normally fertilized oocytes were further considered for embryo transfer or cryopreservation. All zygotes were cultured in cleavage medium (G-M, LifeGlobal, USA or G-1, Vitrolife, Sweden) supplemented with 10% synthetic serum substitute (Irvine Scientific, Santa Ana, CA, USA) up to day 3 after insemination. Prior to transfer or cryopreservation, embryonic development was assessed according to the developmental stage and degree of cytoplasmic fragmentation. Two or three morphologically best embryos were selected for fresh transfer and supernumerary good-quality embryos (seven–eight cells, ≤10% fragmentation) were cryopreserved.

Freezing protocol

A slow freezing protocol with 1,2-propanediol (PROH, Sigma Chemical Co., Sigma-Aldrich, St. Louis, MO, USA) as a cryoprotectant was used [7]. Freezing and thawing solutions consisted of cryoprotectant in phosphate-buffered saline supplemented with 20% *v*/*v* human serum albumin (HSA, LifeGlobal, USA). The freezing procedure was performed at room temperature. Briefly, the embryos were first incubated in 1.5 mol/l PROH freezing solution for 15 min, and then moved to a final solution of 1.5 mol/l PROH+0.1 mol/l sucrose for 15 min and aspirated into the cryostraws. Cooling was carried out in a programmable planar freezer (Kryo ten series; planer products, Sunbury-on Thames, UK) at a rate of −3°C/min to −7°C, at which point seeding was induced manually. Cooling was then continued at rates of −0.3°C/min to −30°C and −50°C/min to −150°C before storage in liquid nitrogen until thaw.

Thawing protocol

On the day of FET, the embryos were thawed rapidly by removing straws from storage, exposed to air for 40 s and immersed in a water bath at 30°C for 1 min. Embryos were sequentially placed in 5-min baths at room temperature with decreasing PROH concentrations (1.0 mol/l, then 0.5 mol/l and finally 0.0 mol/l) while sucrose concentration was kept constant (0.2 mol/l) to remove the cryoprotectant. Thawed embryos were transferred to culture medium at 37°C and evaluated under ×200 magnification for blastomere survival.

Frozen embryo transfer

FETs were performed either in natural monitored cycles or in programmed artificial cycles. Natural cycles were used for women with regular ovulatory menstrual cycles. Thawed embryo transfer was scheduled for 3 days after ovulation. Luteal support was provided with intramuscular injections of progesterone in oil 20–40 mg, from the night of transfer through the 14th day of pregnancy test. For hormone replacement therapy, endometrial development was achieved by oral estradiol. When estradiol levels and endometrial thickness were suitable, this phase was complemented by administration of progesterone. Embryo transfer was performed on the 4th day of progesterone administration. Hormone replacement therapy was continued until pregnancy test. Serum HCG concentration was determined 14 days after embryo replacement and 1 week later. If pregnancy was initiated, steroid supplementation was maintained until week 12 of gestation. Clinical pregnancy was defined as the presence of a gestational sac on ultrasound examination on day 35 after FET. The implantation rate was calculated as the number of gestational sacs identified on ultrasound per number of transferred embryos.

Analysis of parameters

Blastomeres were considered to be damaged when they were lysed, degenerated or dark. Three types of cryopreserved transfer were evaluated to investigate the effect of one–two blastomeres loss on frozen-thawed embryo development: transfers with only fully intact embryos (intact group); transfers with only embryos having lost one–two cells (damage group) and mixed transfers in which one intact embryo and one damaged embryo with one–two cells loss were transferred together (mix group).

Statistics

Statistical analysis was performed using SPSS 10.0. Differences were considered significant if $p<0.05$. For compar-

Table 1 Patient characteristics for the FET cycles

	Intact group	Damage group	Mix group	*p* value
Cycles	104	44	100	
Age (years)	32.29±4.01	32.27±4.91	31.72±5.10	NS
Thickness of endometrium (cm)	1.01±0.12	1.01±0.16	1.01±0.17	NS
Insemination type				
IVF	66	31	72	NS
ICSI	38	13	28	NS
No. of embryos thawed	363	155	348	
No. of embryos transferred (mean ± SD)	190 (1.83±0.57)	80 (1.82±0.72)	200 (2.00±0.00)	–*, **
Healthy blastomeres after thaw/embryo	7.69±0.50	6.71±0.83	7.09±0.87	–*, **, ***
Blastomeres lost after thaw/embryo	0	1.25±0.49	0.60±0.66	

NS Not statistically significant
*$p<0.05$ between intact group and mix group; **$p<0.05$ between damage group and mix group; ***$p<0.05$ between intact group and damage group

ison of the mean variables, Mann–Whitney U test was used. For comparisons of implantation, clinical pregnancy rates and birth rates between the groups a χ^2 analysis was used.

Results

In order to make a valid comparison between intact and partially damaged cryopreserved embryos, we analyzed the data from embryo transfers in which only one embryo or only embryos with equivalent characteristics were transferred. In this retrospective study, we reviewed the outcomes of 248 FET cycles which met the inclusion criteria, including 104 transfer cycles with fully intact embryos (intact group), 44 transfer cycles with damaged embryos alone (damage group) and 100 mixed transfer cycles (mix group). Since only the embryo transfer procedures in which all embryos had one–two blastomeres loss could be included in the damage group, the cases were relatively small ($n=44$). Basic patient characteristics, including mean female age, insemination types (standard IVF or ICSI), mean number of embryos being transferred and endometrial thickness achieved before the progesterone supplementation, did not differ between intact and damage groups (Table 1). Embryo quality before cryopreservation was similar in the three groups. There was a significant reduction of the number of healthy blastomeres and an increase in the number of necrotic blastomeres after thawing in the intact, mix and damage groups, in agreement with their definition (Table 1).

A total of 470 embryos were transferred in 248 transfer procedures, including 290 fully intact embryos and 180 damaged embryos with one–two blastomeres lysis. The outcome of embryo transfer is shown in Table 2. There was no difference between the intact group and the damage group in terms of clinical pregnancy (38.46% vs 38.64%, $p>0.05$), implantation (26.25% vs 26.31%, $p>0.05$) and birth rates (34.62% vs 36.36%, $p>0.05$). These results were also comparable with those obtained with mixed embryo transfers (Table 2).

Discussion

The purpose of this study was to assess the effect of one–two blastomeres lysis on the outcome of frozen embryo

Table 2 Outcome of transferred embryos according to the survival after cryopreservation

	Intact group ($n=104$)	Damage group ($n=44$)	Mix group ($n=100$)	*p* value
Total embryo transferred	190	80	200	
No. clinical pregnancies	40	17	39	
PR per transferred cycle (%)	38.46	38.64	39.00	NS
No. gestational sacs	50	21	52	
IR per embryos transferred (%)	26.31	26.25	26.00	NS
No. deliveries	36	16	35	
Singleton	28	14	22	
Twin	8	2	13	
Birth rate per transferred cycle (%)	34.62	36.36	35.00	NS
No. children born	44	18	48	
Birth rate per embryos transferred (%)	23.16	22.5	24.00	NS

PR Pregnancy rate, *IR* implantation rate, *NS* not statistically significant

replacement cycles. Blastomere loss is a common occurrence in cyrothawed cleavage-stage embryos. Postulated explanations include intracellular ice formation, osmotic damage, metabolic derangements, and the occurrence of cracks within the zona pellucida secondary to freezing. Understanding the impact of such loss on the implantation potential and FER outcome can assist in making clinical decisions on how to best utilize cryopreserved embryos.

It has been documented that the removal of one or two cells from an eight-cell stage embryo does not adversely affect the preimplantation development of such embryos in vitro [8] and in vivo [9], since individual blastomeres of human embryos (until the eight-cell stage) have been shown to be undifferentiated and still totipotent and now this approach is widely used for preimplantation diagnosis of genetic defects. But frozen-thawed embryos with one–two blastomeres loss are different from biopsied embryos where one or two cells have been completely removed. A possible toxic effect from damaged blastomeres on the remaining intact blastomeres has been evoked. Some reports [10, 11] indicated that coexistence of damaged blastomeres and intact blastomeres within mammalian embryos reduces their chances further of developing to term. To compare the development potential between fully intact embryo and damaged embryo with one–two lysed cells, we retrospectively analyzed the outcome of 248 FET cycles. In the present study, initial embryo morphology was similar for all cryopreserved embryos (at the seven–eight cell stage with ≤10% fragmentation). Only those cycles in which all embryos transferred were of the same type were included. The results obtained have failed to detect differences in pregnancy and implantation rates between the two groups selected. This suggested that developmental potential was not obviously affected when the thawed embryo has one–two cells lysed and a remaining six or more intact blastomeres. Our results are in agreement with a previous study [12] in which damaged embryos with less than 25% blastomeres loss were reported to have the same implantation rate as intact thawed embryos.

The toxic effect of damaged blastomeres is highly controversed. Several studies using a mouse model showed that the destruction of blastomeres by micromanipulation dramatically reduced the implantation potential, which were restored when degenerating material was removed microsurgically [11, 13, 14]. A recent study in human embryos [15] demonstrated that removal of necrotic blastomeres shortly after thawing significantly increased the implantation rate of partially damaged thawed embryos. However, only those embryos that have resumed cell division after thawing were transferred in this report. It remains to be determined whether the improvement of the development potential of partly damaged embryos was mainly due to the post-thaw culture selection, or to necrotic blastomere removal. Another study [16] showed dramatically increasing implantation rate in a group that had all lysed blastomeres removed before transfer and suggested that lysed cell removal should be applied routinely. However, mixed embryo transfers in which embryos of different post-thaw quality were replaced in this report. Our data showed that the implantation potential of embryos with one–two blastomeres loss was comparable to fully intact embryos. Therefore, the removal of the necrotic blastomeres seems not be necessary to such embryos. Blastomere loss may influence the outcome only when it exceeds a critical threshold, which is difficult to be defined accurately now. It needs further study to confirm the efficacy of the procedure on embryo with more than 25% lysis.

In conclusion, although Edgar et al. [4] has reported that loss of a minority of cells at early cleavage stages may have an impact on subsequent implantation potential, our results suggest that the presence of one–two lysed blastomeres in the thawed day 3 embryo does not appear to have a negative influence on the further development of the sibling intact cells. Knowledge of blastomere survival to predict subsequent development should assist in devising more rational strategies for the utilization of cryopreserved embryos.

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