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Optimizing an Embryo Cryopreservation Program

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Summary

INTRODUCTION: We analyze 2004 embryo thawing cycles to compare embryo survival, transfer, gestation and implantation rates. MATERIALS AND METHODS: Exceeding embryos scored ≥7 were freezed on day 2, 3 or 4, the rest freezed if reached blastocyst stage. Only evolutive embryos after 24 hours from thawing were transferred. RESULTS: 67 cycles were thawed, 12 day2, 37 day3, 5 day4, and 13 Blastocyst. Surviving rates: 53,6% day 2; 30,2% day 3; 16,6% day 4 and 19% day 5-6. Pregnancy rates: 0% day2; 20% day 3; 50% day 4 and 0% day 5-6. Implantation rates: 0% day 2; 11% day 3; 25% day 4 and 0% day 5-6. CONCLUSIONS: Poor results are found with blastocysts, probably because of their procedence. Day 3 and 4 groups had the best pregnancy and implantation rates, their blastomeres have better evolution and implantation potential after thawing.

Introduction

With the controlled ovarian stimulation, in many patients more eggs than needed for the cycle are obtained and we have exceeding embryos for cryopreservation. The transfer of cryopreserved embryos is less expensive and less invasive for the patient but still have a low implantation and pregnancy rate than transferring fresh embryos. It is a current practice to freeze embryos in different stages of evolution: pronucleate, cleavage embryos, morula and blastocyst.

In our centre, we freeze exceeding embryos as cleavage embryos (day 2, 3 or 4) or as blastocyst (day 5, 6). We studied the results

obtained with the embryo thawing cycles made in 2004 to compare the embryo survival, transfer, gestation and implantation rates and find the optimum day and protocol for embryo freezing.

Materials and Methods

Embryo score

To select the better quality embryos for transfer and cryopreserve, we classify embryos with a score ranging from 0 to 10, attending to: number of blastomeres, degree of fragmentation, blastomeres evenness and presence of multinucleated blastomeres. In our experience (1), the embryos scored 7 to 10 have the best implantation rates and we consider those as good quality embryos.

Day 4 embryos were classified as cleavage-like embryos, early morula, compact and late morula (2).

Blastocysts were classified according to a grade system that values the blastocyst expansion (1,2,3,4), inner cell mass (A,B,C), and trophectoderm morphology (A,B,C). (3).

Freezing protocol

We use propanediol (PROH) as cryoprotectant for embryo freezing in a three step protocol, and glycerol as blastocyst cryoprotectant in a two step protocol. A Planer Series III is used and manual seeding is made at -7ºC for embryos and at -6ºC for blastocyst. (4).

Thawing protocol

A fast thawing protocol is used (4), and the thawed embryos are left in culture overnight (5). Only survival and evolutive embryos are transferred.

Patient's stimulation protocol

All patients had hormonal endometrial preparation with one exception that was a basal cycle. The treatment consisted in 2 mg/day of estradiol valerianate (EV) from day 3 of the cycle during 5 days, 3 days with 4 mg/day and from day 11, 6 mg/day. The day before embryo thawing or 4 days before blastocyst thawing, supplementation with 400-600 mg/day of micronized progesterone vagynally was done. ßhCG was measured 20 days after embryo transfer.

Results

In day 4 group, one patient had 5 late morula freezed that did no

Table I

	N°Patie.	N°Pa.Tra(%)*	N°Em	N°E.Surv(%)ª	N°Gest	%Gest	%Imp
Day2	12	9 (75)	41	22 (53,6)	0	0	0
Day3	37	20 (54)	149	45 (30,2)	4	**20**	**11**
Day4	5	2 (40)	24	4 (16,6)	1	**50**	**25**
Blast.	13	4 (30,7)	32	6 (19)	0	0	0
Total	67	35 (52,2)	246	77 (31,3)	5	14,2	2,4

*N° patients that had embryo transfer.
ªN° surviving embryos.

survived, two patients had only cleavage embryos 8-10 cells that survived partially but had no transfer because had no evolutive, and in the remaining two patients had at least one compacting morula that survived and 24 hours later had a transfer with late morula or expanding blastocyst. The patient with the expanding blastocyst transfer became pregnant.

One of the pregnancies obtained in the day 3 group was the patient with the basal cycle, other pregnancy on day 3 was a twin that reduced at single in the first trimester of gestation, and a third pregnancy on day 3 had a miscarriage. Three pregnancies are ended with healthy babies and one is in course.

Conclusions

Each embryonic stage has qualities that make it the right stage for freezing; however, each stage also has its disadvantages that limit its success rate. In our unit we use the blastocyst program only for poor implanters, in the larger group of patients, we transfer on day 2 or 3, and cryopreserve exceeding embryos the same day of transfer if the embryos have good quality, and the poor quality exceeding embryos are left in culture until day 5 or 6. Only the blastocyst reached from the last ones are feezed. The poorer results are obtained with freezed-thawed blastocyst with the lower transfer rate per patient. One cause may be the procedence of the blastocyst, exceeding embryos scored below 7 that develop in culture, but probably are not the better quality blastocyst for freezing. Other authors (6) find improved results changing the freezing protocol, lowering the start temperature and cooling rate. Using a reduced starting temperature and faster cooling rate, blastocyst were exposed to the cryoprotectant for less time, and this can have a significant importance because of the known toxicity of cryoprotectants. It is important to mention the source or derivation of blastocysts because has to maintain two different cell types, the inner cell mass and the trophectoderm cells, and this cells may have different permeability to water

and cryoprotectant. Also, lower pregnancy rates are reported with fresh blastocyst transfer with day 6 transfer compared with day 5 transfer (7), and in all but two of our freezed and thawed blastocysts patients, we freezed on day 6.

Day 2, 3 and 4 groups had the same embryo freezing protocol with PROH, but the results are quite different. Embryos freezed on day 2 have a good survival rate but a poor implantation rate. Developmental and implantation potential in embryos on day 2 can be related with blastomeres loss (8),(9) and we may improve those embryos selection by leaving them in culture until blastocyst stage.

Day 3 embryos have an acceptable survival and pregnancy rate; apparently surviving blastomeres have better developmental potential at this stage.

Other groups that freeze embryos at the morula stage perform transfer 1-2 hours after thawing and apply assisted hatching to all embryos regardless of embryo quality (2). In our serie, day 4 patients with embryo transfer were the ones with at least one compacting morula freezed. When thawed, those embryos showed cell separation and the following day showed at the time of transfer, full compaction or blastocyst expansion. In our experience, better results of survival and implantation rates are obtained when day 4 cryopreservation is performed at the stage of compaction and before late morula stage.

According to those results seems that blastomeres surviving from embryos more divided have better potential for evolution and implantation after thawing. It is known that the number of good-quality embryos decreases daily, so the earlier the embryonic stage, the more uncertain the embryo viability. After this study of 2004 results, we will preferentially freeze exceeding embryos on day 3 or 4.

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