

## Comparison of Survival Rates Between Frozen Thawed and Fresh Embryos After Embryo Biopsy Using Acid Tyrode's Solution\*

Lim Mui Nee, Christine Yap *FRANZCOG, MRCOG*, Lim Tse Hui *Bsc (Hons), Msc*, Yong Yin Yin *BSc*,  
Lee Shaw Ni Amy *Bsc (Hons)*, Lim Soon Tiong Alvin *PbD, HGSAAC*,<sup>1</sup> Yu Su Ling *FRCOG, FAMS*

Centre for Assisted Reproduction, Department of Obstetrics and Gynaecology, SGH

<sup>1</sup>Department of Pathology, SGH

### ABSTRACT

**Background.** Preimplantation genetic diagnosis (PGD) is a method of prenatal diagnosis, where medical tests are used to find out about potential genetic abnormalities in the embryo before implantation. PGD involves the biopsy of blastomeres from embryos derived from in-vitro fertilisation (IVF). The technique of biopsy involves “drilling” a hole through the zona pellucida using Acid Tyrode's solution and the aspiration of 1 to 2 blastomeres with a biopsy pipette. So far, there is limited data on embryo survival after human embryo biopsy. In this study, we aimed to compare the survival and blastulation rates of frozen thawed and fresh embryos after embryo biopsy prior to genetic analysis by polymerase chain reaction (PCR) or fluorescent in situ hybridisation (FISH).

**Methods.** Human embryos, which had been discarded after IVF or intracytoplasmic sperm injection treatment due to poor embryo quality or abnormal fertilisation, and frozen thawed embryos of good quality (Grade 1 or 2) for which patients had given consent for research purposes, were used for this study. Embryo biopsy was performed in calcium-magnesium free media under paraffin oil. Using an inverted microscope with a micromanipulator, Acid Tyrode's solution was used to dissolve a small hole in the zona pellucida of the embryo. A 30mm biopsy needle was then used to aspirate the blastomeres that were then prepared for further analysis.

**Results.** The results showed that the survival rate after embryo biopsy for frozen thawed embryos and fresh embryos was 76.6% and 66.6%, respectively. The blastulation rate of the frozen thawed good grade embryos was 33.3% while that for fresh poor grade embryos was only 8.3% ( $p < 0.05$ ).

**Conclusion.** Human embryos had good survival rates after biopsy and those with good morphological quality had higher blastulation rates. However, those with poor morphology reflecting abnormal development were more likely to arrest after biopsy.

*Keywords:* blastomeres, embryo biopsy, preimplantation genetic diagnosis, survival rate

### INTRODUCTION

The first clinical application of preimplantation genetic diagnosis (PGD) was described by Handyside *et al.*<sup>1</sup> Since 1990, the number of indications for which PGD has become possible has increased continuously,<sup>2</sup> and with the help of sensitive molecular methods, genetic analysis at the single-cell level can be done. Several approaches to obtain material for genetic analysis in PGD have been proposed.

Many women in in-vitro fertilisation programmes (IVF) are over 35 years of age and at an increased risk of chromosomal abnormalities, particularly aneuploidy resulting during meiosis or initial cleavage. By identifying abnormal embryos and transferring only chromosomally normal embryos, implantation rates should increase while spontaneous abortions and trisomic pregnancies should decrease. In addition, PGD may prove to be useful in the investigation of unexplained IVF failures in young patients.

PGD involves the biopsy of blastomeres from embryos at the 5 to 8 cell stage derived from IVF. The technique of biopsy involves “drilling” a hole through the zona

---

\* Presented at the SGH 13th Annual Scientific Meeting on 26–27 April 2002.

Table 1. Outcome of survival and blastulation rates after biopsy of frozen thawed embryos and fresh embryos.

	Frozen thawed embryos without biopsy (control)	Frozen thawed with biopsy	Fresh embryos without biopsy	Fresh embryos with biopsy (control)
Number of embryos	22	30	19	25
Embryos survival rate (%)	95	76.6	73.7	66.6
Blastulation rate (%)	36.4	33.3	10.5	8.3

Blastulation rate of frozen-thawed biopsied embryos vs fresh embryos biopsied: P=0.024.

pellucida using Acid Tyrode's solution and the aspiration of 1 to 2 blastomeres with a biopsy pipette. Extensive research has demonstrated that this technique of embryo biopsy has no effect on the ability of embryos to continue normal growth and development. The two biopsied cells are used for genetic diagnosis.<sup>1</sup> So far, there is limited data on embryo survival after human embryo biopsy.

In this study, we aimed to compare the survival and blastulation rates of fresh and frozen thawed embryos after embryo biopsy prior to genetic analysis for unbalanced chromosome arrangements or DNA mutation that cause single gene disorders by using polymerase chain reaction (PCR) or fluorescent in situ hybridisation (FISH).

## METHODS

This study had been approved by the Ethics Committee of the Singapore General Hospital. Human embryos, which had been discarded after IVF treatment due to poor embryo quality or abnormal fertilisation and frozen thawed embryos of good quality (Grade 1 or 2) for which patients had given consent for research purposes, were used for this study.

### *Biopsy Methods*

Three 30ml droplets of calcium-magnesium free media EB-10 (IVF Science) were introduced in the middle of a culture dish (Falcon) and shaped into an oblong droplet. The droplets were then covered with 3.5ml of paraffin oil (MediCult). The dishes were incubated at 37°C with 5% CO<sub>2</sub>.

Using an inverted microscope (Nikon, Japan) with a micromanipulator (Narishige, Japan), pipettes were mounted on the microscope in a similar way when performing intracytoplasmic sperm injection. The holding pipette (Research Instruments IH35) was placed on the left-hand side. The drilling and biopsy pipette (Research Instruments, UK) were placed on a

double tool holder (Narishige HD-21, Japan) on the right hand side. The drilling and biopsy pipette were positioned in the same plane with the opening in one line. This allowed visualisation of either pipette by moving them along the Y-axis.

Two embryos, one in each droplet, were placed in one dish. The outer droplets were used for rinsing. A droplet of acid tyrode solution (Sigma) was placed on the cover of the dish, the drilling pipette lowered into the droplet and acid tyrode aspirated. The embryo dish was placed on the heated stage of the microscope and an embryo was fixed on the holding pipette. The embryo was rotated so that the blastomeres were located at the site where drilling was to be performed.

The drilling pipette was lowered close to the zona pellucida at the 3 o'clock position and acid tyrode was released until the acid ruptured a hole on the zona. The drilling pipette was moved out of the field and the biopsy pipette placed in front of the opening in the zona. The biopsy pipette could freely pass through the opening towards the nearest blastomere. Only blastomeres containing a nucleus were aspirated slowly into the pipette. The embryo was transferred into culture medium G2.2 (IVF Science) and the blastomeres were treated further depending on the procedure used for genetic diagnosis.

## RESULTS

Fifty-two frozen thawed embryos with good morphological quality and 44 fresh embryos with poor morphological quality were divided into 4 groups: frozen thawed embryos without biopsy (good morphological quality control group), frozen thawed embryos with biopsy (good morphological quality group), fresh embryos without biopsy (poor morphological quality control group) and fresh embryos with biopsy (poor morphological quality group).

The survival rate in the biopsy group for frozen thawed embryos with good morphological quality was 76.6%, whereas that for fresh embryos with poor morphological quality was 66.6%. The survival rate in the control group (without biopsy) was 95% and 73.7% for frozen thawed embryos with good morphological quality and fresh embryos with poor morphological quality, respectively. The blastulation rate in the biopsy group of the frozen thawed embryos with good morphological quality embryos was 33.3%, while that for fresh embryos with poor morphological quality was only 8.3% ( $p < 0.05$ ). The blastulation rate in the control group was 36.4% and 10.5% for frozen thawed embryos with good morphological quality and fresh embryos with poor morphological quality, respectively (Table 1).

## DISCUSSION

The list of genetic disorders for which PGD is performed is undergoing rapid extension and currently includes more than 2 dozen conditions. In addition, there has been interest in PGD for late-onset disorders and those conditions that have never been considered acceptable for prenatal diagnosis. As pre-selection of oocytes and embryos does not involve termination of pregnancy, but rather allows a normal pregnancy to be established, it provides an important option for those couples who have religious or ethical concerns regarding currently available techniques for prevention of genetic disorders.

PGD has also become an integral part of assisted reproduction by avoiding the transfer of chromosomally abnormal and potentially non-viable embryos. This may contribute in future to a significant increase in the implantation and pregnancy rates in IVF and to a general improvement of the standards of assisted reproduction practice.

The removal of 1 or 2 blastomeres does not affect further development of the embryo<sup>3</sup> and, in contrast to earlier stages, the cellular mass of the biopsied embryo is not reduced too much by the biopsy procedure.<sup>4</sup>

## CONCLUSION

In this study, it can be concluded that human embryos have good survival rates after biopsy and those with good morphological quality have higher blastulation rates. However, embryos with poor morphology reflecting abnormal development are more likely to arrest after biopsy. Embryo biopsy and blastomere fixation were therefore performed on embryos based on their morphological evaluation and selection.

Embryos with poor morphological quality due to an absent or damaged nucleus should not be selected for further analysis as they might give false results or no result.

## ACKNOWLEDGEMENTS

I would like to thank Miss Chen Siew Gian for the embryological skills. I would also like to thank Miss Lim Siew Li, Miss Hum Siew Chen and Miss Carine To Chiou Fen for editorial assistance.

## REFERENCES

1. Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature* 1990; 344:768-70.
2. Lissens W, Sermon K. Preimplantation genetic diagnosis: current status and new developments. *Hum Reprod* 1997; 12:1756-61.
3. Hardy K, Martin KL, Leese HJ, Winston RM, Handyside AH. Human preimplantation development in vitro is not adversely affected by biopsy at the 8-cell stage. *Hum Reprod* 1990; 5:708-14.
4. Tarin JJ, Handyside AH. Embryo biopsy strategies for preimplantation diagnosis. *Fertil Steril* 1993; 59:943-52.