

The Impact of Cleavage Stage Biopsy on Blastocyst Development

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Objective: To evaluate the impact of blastomere biopsy on mouse embryos at cleavage stage and the cofactors that may affect the blastocyst development.

Design: An Experimental Animal Study.

Setting: Department of Obstetrics and Gynecology, University of Monash, Melbourne, Australia.

Method: Cleavage stage of mice embryos at four cells (group 1) and eight cells were biopsied; the eight-cell embryos either had one cell (group 2) or two cells removed (group 3). The effect of biopsy on the embryo was compared with the control group (group 4, no biopsy).

Result: The rate of hatched blastocyst was significantly better in group 4 (77.27%) compared to group 1 (50%) (P-value=0.0023) and similar to group 2 (66.7%) and group 3 (64 %). There was no significant impact on the number of blastomeres removed in eight-cell stage and blastocyst development. The overall hatched blastocyst rate was significantly lower in the biopsied embryos incubated in the Ca^{2+}/Mg^{2+} free medium for more than 15 minutes, P-value=0.0455.

Conclusion: Biopsy of cleavage-stage embryos at eight-cell stage have less adverse impact on blastocyst viability compared to biopsy at four-cell stage. The incubation of embryos in Ca^{2+}/Mg^{2+} free medium for more than 15 minutes reduced blastocyst development.

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The pre-implantation genetic diagnosis (PGD) is an integral procedure of assisted reproduction technology since its introduction in 1990. It has been developed as a screening and diagnostic technique for patients at risk of transmitting genetic diseases to their offspring.

The PGD and pre-implantation genetic screening (PGS) techniques facilitate the genetic investigation of the human embryo by either biopsy of polar bodies from oocyte at the day of retrieval or day 1 of fertilization, biopsy of one or two blastomeres from cleavage stage embryo, and of trophoblast cells from blastocyst stage¹⁻³.

Cleavage stage biopsy of one or two blastomeres remains the preferred practice for PGD, accounting for approximately 90% of the reported cases^{1,4}. Up to date, no agreement has been reached on how many blastomeres should be biopsied for genetic investigation. Studies reported adverse effects on the embryo development and pregnancy rate subsequent to the removal of two blastomeres^{5,6}. In contrast, van de Velde et al suggested that biopsy of one or two cells at the cleavage stage (8 cells) does not affect the implantation or the pregnancy rate, with the advantage of improving the efficacy of the diagnostic tests^{7,8}.

The standard IVF culture medium can be used for the biopsy during PGD. However, its effectiveness depends on the development stage of the embryo¹. Embryos at cleavage stage

of 6-8 cells which showed compaction loosened the blastomeres from the membrane when incubated in Ca^{2+}/Mg^{2+} free medium for a short period; the effectiveness of this practice was reported by Dumoulin J et al and suggested no adverse outcome on the embryo development^{8,9}. This is becoming a common practice for biopsy at cleavage stage^{1,7,8}. However, others suggested that by incubating the embryo in such a medium could compromise the embryo's survival ability¹⁰.

Cleavage stage biopsy is an invasive test which evaluates the genetic composition of the embryo; the procedure carries adverse effects on the embryo development. The benefit of the procedure outweighs the risks involved.

The aim of the study is to evaluate the optimal embryo developmental stage for biopsy and the impact on the embryo development.

METHOD

Female F1 mice (CBA X C57B16/J) of approximately 5-7 weeks old were super-ovulated. One hundred twelve two-cell embryos were collected and cultured for 24 hours in a humidified CO₂ incubator at 37°C under 5% v/v. The following day, the embryos were evaluated and grouped according to cell number; only 4-8 cell embryos were included in the study. The embryos were evenly and randomly allocated to the four study groups, see figures 1 and 2.

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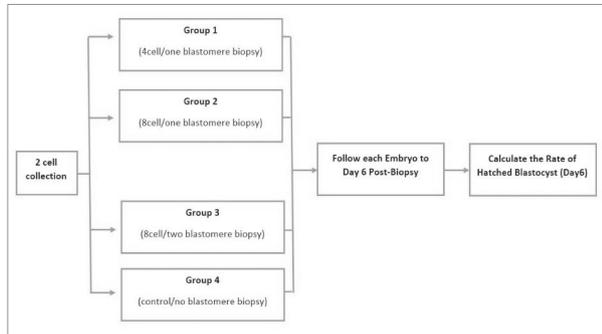


Figure 1: Study Design

Embryos were de-compacted in Ca^{2+}/Mg^{2+} free biopsy media. The total time of incubation in the biopsy media was less than 15 minutes. A research instrument (RI) stage micromanipulator with non-contact Saturn laser system was used in the study (Cornwall, England). After the biopsy, the embryos were cultured in a cleavage medium (Cook Medical). All embryos were transferred to blastocyst medium (Cook Medical, Australia) 90 hours post-hCG injection, and continued recording their development up to day six post-insemination, see figure 2.

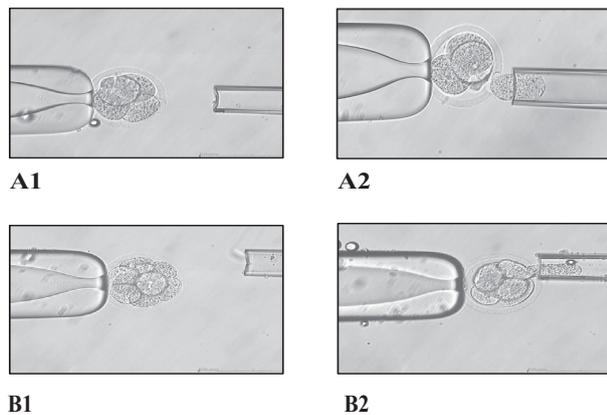


Figure 2: (A1-A2) Biopsy of 4-Cell Mouse Embryo, (B1-B2) Biopsy of 8-Cell Mouse Embryo

Embryos of the control group (group 4) were transferred to the biopsy medium for ≤ 15 minutes and then treated as the biopsied embryos.

Statistical analysis was performed using SPSS, version 20.0. A Pearson's Chi-square test (X^2) was used for statistical comparisons between the groups. A P-value of <0.05 was considered significant.

RESULTS

One hundred twelve 2-cell embryos were collected randomly and were evenly divided between the four groups. Twenty-eight (25%) 4-cell embryos had one cell removed, and 52 (46%) 8-cell embryos either had one or two cells removed. The remaining 22 (19.6%) embryos that were not manipulated (no biopsy) were used as control; 10 embryos were excluded due to poor quality, see table 1.

Table 1: Number of Embryos Biopsied and the Rate of the Hatched Blastocyst in the Four Groups

Research Group	Number of 2-cell collected	Number of embryo suitable for biopsy	Number of embryo survived after biopsy	Number of grade-6 blastocyst on Day 6	The rate of hatched blastocyst (Grade 6)
Group 1 (4cell/1cell biopsied)	28	28	28	14	50%
Group 2 (8cell/1cell biopsied)	28	26	25	16	64%
Group 3 (8cell/2cell biopsied)	28	26	24	16	66.66%
Group 4 control (no biopsy)	28	22	-	17	77.27%

No significant difference was discovered between one or two blastomeres removed from the 8-cell embryos group 2 and group 3, 16/25 (64%) and 16/24 (66.7%), respectively. However, a significant difference was discovered in the rate of hatched blastocysts between group 1 (4-cell) and group 4 (control), 14/28 (50%) and 17/22 (77.3%), respectively, P-value=0.0023. A significant difference was also discovered between the 4-cell group and both 8-cell groups, P-value=0.0455, see table 1 and figure 3.

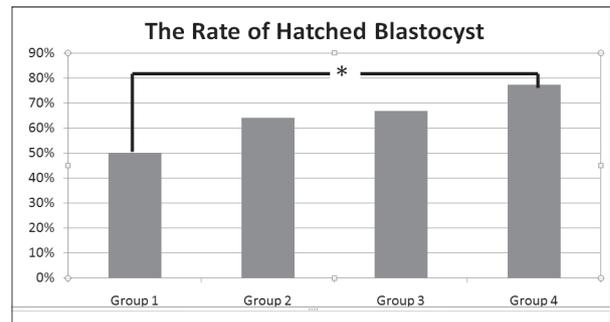
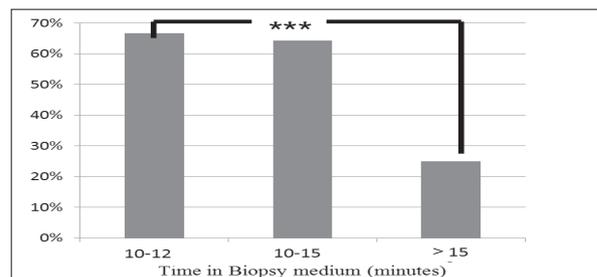


Figure 3: The Rate of Hatched Embryo on Day 6 per Group, P-Value*= 0.0023

The total incubation time of the embryos in the Ca^{2+}/Mg^{2+} free media of more than 15 minutes had a significant impact on the rate of the hatched blastocyst, see figure 4. Only 1/4 (25%) of the embryos hatched when incubated in the biopsy medium for more than 15 minutes compared to 57/86 (66%) and 8/12 (67%) embryos when incubated for less than 15 minutes (P-value=0.0455).



***P-value=0.045

Figure 4: The Rate of the Hatched Blastocyst in Relation to Time Spent in The Biopsy Medium

DISCUSSION

PGD is used to detect disorders and genetic conditions¹¹. It is based on the concept that embryos at early stages have blastomeres, which are totipotent^{12,13}. Therefore, every cell is an equal indicator of the whole. However, the innocence of the procedure still could not be assumed, as removal of nucleated blastomere from the embryo is a random biopsy and may upset the cellular organization of the embryo¹⁴. The effect demonstrated in this study was with the reduction of the rate of the hatched blastomeres in the four-cell embryo. However, no statistically significant difference was discovered in the rate of hatched embryos between the control group and 8-cell biopsied embryo groups; the result is similar to previous studies which failed to detect any adverse effect of the biopsy on 8-cell embryo viability and blastocyst development in-vitro^{7,15}.

Incubating the embryo in Ca²⁺/Mg²⁺ free medium facilitates the decompaction process, and makes the biopsy easier¹⁶. In this study, it was found that embryos incubated for more than 15 minutes in the biopsy medium caused a reduction in the hatched blastocyst rate (25%) compared to the hatched rate in embryos incubated for less than 15 minutes (66%). The incubation time was similar to the clinical incubation time in PGD laboratories. It could be due to the small sample size (n=77) or the exposure of the embryos to the different culture media causing epigenetic or imprinting changes that may impact embryo development and clinical outcomes¹⁷⁻²⁰.

A study found that up to 40 minutes in the biopsy medium would not have any adverse effect on the viability of the embryo²¹. Another study found no loss of viability of the biopsied embryos after incubation of up to 90 minutes²². Further assessment is required for the effect of the biopsy medium on the viability of the embryo. One of the main limitations of the study is the small sample size, which may have an impact on the validity of the results.

CONCLUSION

The best time for cleavage stage biopsy is at the eight-cell stage and incubation period in the biopsy medium should be less than 15 minutes to reduce the adverse effect on embryo viability.

Randomized controlled studies with the use of in-vitro mouse embryos to validate the optimal biopsy stage are recommended. In addition, evaluation of the impact of blastomere biopsy on human embryo at cleavage stage and the different cofactors that may have an effect on the blastocyst development are recommended.

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