

The effect on cleavage of two-cell mouse embryos after a delay in embryo retrieval in a human *in vitro* fertilization programme

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Summary

Two-cell mouse embryos are used for quality control in a human *in vitro* fertilization programme. A controlled experiment was designed to evaluate the effect on cleavage of two-cell mouse embryos after a delay in embryo retrieval. In the test group, two fallopian tubes were incubated in Whittingham's T6 medium for 2½ hours per experiment before the embryos were removed for culture. In the control group embryos were removed from the fallopian tubes immediately after the mice were sacrificed. Five experiments were performed. Eight of 141 two-cell embryos (5,7%) reached the blastocyst stage in the test group, and in the control group 143 of 151 two-cell embryos (94,7%) reached the blastocyst stage after 72 hours. Embryos must be removed immediately after the mice are sacrificed to obtain constant results. If not, poor cleavage can lead to unnecessary confusion in the laboratory.

S Afr Med J 1985; 68: 743-744.

The mouse oocyte system is used routinely for quality control in human *in vitro* fertilization laboratories.¹ The two-cell mouse embryo can give an indication of toxic substances in the growth medium² and, although not infallible, the method is of great benefit in testing new equipment and the medium weekly.

However, it is important to know that this system can give false results leading to unnecessary confusion in the laboratory. One factor, the time interval between obtaining the fallopian tube and retrieval of the embryos, which can lead to poor cleavage of two-cell mouse embryos, is dealt with.

Method

Whittingham's T6 medium was freshly prepared weekly in a laminar airflow cabinet. The osmolarity was adjusted between 280 and 283 mmol/kg/l. Glassware (Pyrex) was washed and sterilized as described by Whittingham.³ The medium with

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20% human serum was incubated in a CO₂-in-air incubator (Forma Scientific 3163) for 24 hours, when the pH was 7,4 (pH meter M83 autocal). The serum was prepared by the method described by Leung *et al.*⁴

F1 female mice (C57 B1/6 x CBA) were prepared for superovulation as discussed in a previous publication.⁵ They were sacrificed by cervical dislocation 45 hours after human chorionic gonadotrophin 10 IU had been injected intraperitoneally.

The fallopian tubes were removed and one was put into a tissue culture tube (No. 1) filled with 2 ml of gassed medium (T6). The second fallopian tube from the same mouse was put into bottle No. 2 with the same amount of medium. Both tissue culture bottles (Falcon 2058) were immediately put back into the incubator. After the second mouse had been sacrificed the same procedure was repeated.

Embryos were obtained from the fallopian tubes by the method described by Gates.⁶ The fallopian tubes in bottle No. 1 were explored immediately after removal from the mouse and the embryos obtained were put into a Petri dish (Falcon 3037) filled with growth medium. The Petri dish with embryos was incubated for 72 hours.

Tissue culture bottle No. 2 was left in the incubator for 2½ hours before the embryos were removed. The embryos were also pipetted into a culture dish (Falcon 3037) and subjected to the same conditions as No. 1 for 72 hours.

After 72 hours the number of two-cell embryos in each Petri dish which had cleaved to the blastocyst stage were evaluated. It was not known before evaluation to which group the embryos belonged. The experiment was repeated 5 times.

Results

After 72 hours the number of two-cell mouse embryos that reached the blastocyst stage was noted (Table I). In the test group only 8 of the 141 two-cell embryos (5,7%) reached the blastocyst stage but in the control group 143 of 151 two-cell embryos reached this stage (94,7%). The difference in cleavage was statistically significant ($P < 0,0001$; chi-square test).

TABLE I. NUMBER OF BLASTOCYSTS AFTER 72 HOURS

Experiment	Test group	%	Control group	%
1	0/29	0	25/25	100
2	5/26	19,2	35/35	100
3	0/23	0	19/20	95
4	0/13	0	34/40	85
5	3/50	6	30/31	96,8
Total	8/141	5,7	143/151	94,7

Discussion

Two-cell mouse embryos are used twice a week for quality control in the human *in vitro* fertilization programme at Tygerberg Hospital. If cleavage to the blastocyst stage fails to reach the 90% level after 72 hours this can be an indication of suboptimal culture conditions.

Poor cleavage as the result of a time interval between removal of the fallopian tubes and recovery of the embryos was discovered as the result of good documentation of the mouse experimental work. A controlled study was planned to evaluate this observation because poor cleavage led to confusion in the laboratory. These poor results also cause unnecessary preparation of fresh medium and evaluation of the culture conditions in a search for a defect in the system.

An explanation for the poor cleavage in the test group could be the development of a low oxygen concentration in the fallopian tube after surgical removal. Mouse embryos fail to develop in the absence of oxygen or when less than 0,56% is present.⁷

It is of the utmost importance to follow a strict protocol in human *in vitro* fertilization programmes. This simple problem of the time interval also shows that the same strict protocol must be followed with the mouse oocyte quality-control system. Embryos must be obtained immediately after the fallopian

tubes have been removed otherwise there will be poor cleavage, and a wild goose chase in the laboratory.

The authors wish to thank Sister H. Rosich for her help as research assistant, Mrs L. Brand and Mrs H. Krüger for the preparation of this manuscript, and Mr P. Africa for his help in the mouse laboratory.

This article is based on an M.D. thesis at the University of Stellenbosch under the guidance of Professor H. J. Odendaal.

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The effect of fluorescent light on the cleavage of two-cell mouse embryos

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Summary

Two-cell mouse embryos were subjected to fluorescent light, 2900 lux, for 30 minutes, and the cleavage compared with that in a control group. There was no statistically significant difference in the results. In both groups 90% of two-cell embryos reached the expected level of cleavage. The possible effect of fluorescent light on the oocyte is discussed.

S Afr Med J 1985; **68**: 744-745.

The effect of fluorescent light on the cleavage of embryos and specifically two-cell mouse embryos was an unanswered question in the *in vitro* fertilization (IVF) unit at Tygerberg Hospital when we started with the preliminary work on human IVF. Purdy¹ stated that tungsten bulbs are preferable in the laboratory to avoid emission from fluorescent lighting. Short-wavelength visible light is detrimental to unfertilized hamster eggs in that prolonged exposure disturbs the completion of normal meiosis.²

A controlled study to evaluate the effect of fluorescent light on the cleavage of two-cell mouse embryos to the blastocyst stage was carried out.

Method

F1 female mice (CBA x C57 B1/6) were prepared for super-ovulation as outlined previously.³ The mice were sacrificed 45 hours after 10 IU human chorionic gonadotrophin (HCG) had been injected intraperitoneally. Only 2 of 4 mice were sacrificed at a time. The fallopian tubes were obtained, put into Whittingham's T6 medium plus 10% human serum previously gassed

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