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.

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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, 2 900 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.

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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. Purdy[1] 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

in a 5% CO_2-in-air incubator (Forma Scientific) for 24 hours. At that stage the pH of the medium was 7,4.

The first part of the experiment was performed under fluorescent light in a laminar airflow cabinet. The embryos were pipetted into a 3037 Falcon Petri dish filled with 10% serum and 90% medium, and incubated for 72 hours.

The amount of light in the laminar airflow cabinet was measured in the exact spot where the embryos were handled. The measurements were performed with a lux meter (Thorn EMI Lighting, Enfield, UK, type L105-14, range 20 - 20 000 lux). The measurements were as follows: dissecting microscope alone — 690 lux; dissecting microscope plus fluorescent light in laminar airflow cabinet (Atlas 55W (B3.1) Coolwhite (colour 2)) — 2 900 lux.

The embryos were subjected to 2 900 lux for 30 minutes. Distance from light source to the embryos was 65 cm.

The second part of the experiment followed immediately. The second 2 of the 4 mice were sacrificed and the fallopian tubes explored for two-cell embryos. This part of the procedure was performed within the laminar airflow cabinet without fluorescent lighting. The embryos were subjected to only the microscope light (690 lux) for 30 minutes.

The amount of embryos reaching the blastocyst stage was evaluated after 72 hours. The authors did not know which embryos belonged to the test group and which belonged to the control group. The experiment was repeated three times.

Results

Cleavage of two-cell mouse embryos to the blastocyst stage is shown in Table I. Cleavage to the blastocyst stage in the test group was 92,6% and in the control group 92%. There was no statistically significant difference between the two groups; both reached the 90% level of cleavage.

TABLE I. CLEAVAGE OF TWO-CELL MOUSE EMBRYOS TO BLASTOCYST STAGE

Experiment	Test group	%	Control group	%
1	10/10	100	10/10	100
2	39/43	90,7	33/36	91,7
3	26/28	92,9	26/29	89,6
Total	75/81	92,6	69/75	92,0

Discussion

In this study cleavage of the two-cell embryos was not affected by exposure to fluorescent light for 30 minutes. Hirao and Yanagimachi[2] pointed out clearly that short-wavelength visible light (< 470 - 480 nm) from ordinary light sources is detrimental to unfertilized hamster eggs, in that prolonged exposure to the light disturbs the completion of normal meiosis after the eggs have been penetrated by spermatozoa. The fluorescent light commonly used in modern laboratories is more harmful than the light from incandescent lamps. They recommended red cellophane sheets to protect eggs against harmful effects.[2]

It is interesting to note that the time of exposure to fluorescent light is very important. In 84,5% of eggs, meiosis was normal after irradiation for 15 minutes, but in only 16% of eggs did the process develop normally after exposure for 30 minutes.[2]

The IVF group working in Norfolk, Va, USA, perform routine photography without any obvious effect on cleavage of human oocytes and with good pregnancy results.[4] An explanation for the high rate of cleavage could be the fact that the irradiation time with photography is very short. This statement is supported by the finding noted above[2] that with irradiation of up to 15 minutes with fluorescent light, the effect on meiosis in the hamster egg is also minimal.

Although the detrimental effect of light on two-cell mouse embryos could not be detected, notice should be taken of the findings of Hirao and Yanagimachi[2] and irradiation with any light source restricted to a minimum, especially on unfertilized eggs because of the possible effect on the completion of meiosis.

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