Mouse embryos cultured in amniotic fluid

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Summary

One thousand mouse embryos were collected over a period of 6 weeks and randomly assigned to either amniotic fluid aspirated during routine amniocentesis from normal 16 weeks pregnant patients or Earle's medium. The embryos were cultured for 72 hours at 37°C in 5% carbon dioxide in air. Osmolarity, pH, partial arterial carbon dioxide pressure and HCO_3^- were checked before culture and again at the end of culture. Embryos were assessed according to the stage of development, namely degenerate embryos, morulae and blastocysts. The blastocysts were further divided according to their stage of development. There was a significant improvement in blastulation rate to fully expanded blastocysts in the embryos cultured in the amniotic fluid. The amniotic fluid was fairly variable in composition, pH and osmolarity, whereas the Earle's medium was very uniform in composition between trials. It was concluded that amniotic fluid drawn at 16 weeks from normal women is a good culture medium for mice embryos.

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Mouse embryos are used as a monitor of human *in vitro* fertilisation (IVF) culture conditions. Embryo culture media are often complicated solutions requiring careful monitoring to ensure uniformity for successful embryo culture.[1-3] Much of the quality control is outside the scope of the IVF laboratory, for example the source of original chemicals and conditions during distribution.

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The successful use of human amniotic fluid as a culture medium for mouse and human embryos has been reported.[4] At Groote Schuur Hospital there is a constant supply of 16-week amniotic fluid which is drawn for the detection of fetal abnormalities. It was felt that this should be tested as a possible alternative culture medium for mouse embryos and ultimately human embryos.

Materials and methods

Amniotic fluid was aspirated from normal patients during routine amniocentesis at 16 weeks. These samples were centrifuged at 800 *g* for 30 minutes and the supernatants filtered through 0,2 μm Millipore filters. The fluid was stored at 4°C until use, but not longer than 1 week. Earle's medium (GIBCO, Grand Island, NY) supplemented with 10% deactivated fetal cord serum was used as control. Mice were superovulated by conventional methods and mated naturally.[3] Two days after the injection of human chorionic gonadotrophin the mice were killed by cervical dislocation, the fallopian tubes were dissected out and the embryos then flushed into a dish containing Earle's medium. Thereafter, the embryos were assigned at random to either another dish of Earle's medium or to amniotic fluid. Osmolarity, pH, partial arterial carbon dioxide pressure ($PaCO_2$) and HCO_3^- (ABL3 blood gas analyser, Radiometer, Copenhagen) were checked on both media before and after culture. After 72 hours' culture at 37°C under an atmosphere of 5% carbon dioxide in air, the embryos were assessed according to their stage of development, namely, degenerate embryos, morulae and blastocysts. The blastocysts were further divided into: stage A (blastocyst cavity just beginning, with the cavity comprising approximately 25% of the embryo volume); stage B (blastocyst cavity approximately 50% of the embryo); stage C (blastocyst cavity 75% of the embryo); and stage D (fully expanded blastocyst). The experiment was conducted weekly for 6 weeks, until a total of 500 embryos had been assigned to each group.

Statistical analysis. The difference between embryo growth in amniotic fluid and Earle's medium was assessed by two-way

TABLE I. TWO-WAY ANALYSIS OF VARIANCE FOR MOUSE EMBRYOS GROWN IN EARLE'S MEDIUM OR AMNIOTIC FLUID

			Blastocyst stage			
No.	Degenerate	Morulae	A	B	C	D
Earle's medium	**60**	**53**	**35**	**18**	**43**	**291**
Amniotic fluid	**49**	**6**	**11**	**11**	**30**	**393**
$F_{1,5}$	**1,282**	**5,542**	**6,151**	**3,374**	**3,261**	**15,963***
P	**0,309**	**0,0652**	**0,0558**	**0,126**	**0,131**	**0,0104***

*Denotes significance.
$F_{0,05,1,5} = 6,61$
$F_{0,001,1,5} = 16,3$

TABLE II. COMPOSITION OF EARLE'S MEDIUM AND AMNIOTIC FLUID BEFORE AND AFTER 72 HOURS' CULTURE

	Earle's Medium		Amniotic fluid	
	Before	After	Before	After
pH	**7,385 ± 0,05**	**7,342 ± 0,023**	**7,285 ± 0,088**	**7,268 ± 0,069**
mOsm/kg	**282,167 ± 0,753**	**283,667 ± 2,066**	**282 ± 2,828**	**297,667 ± 16,342**
HCO_3^-	**21,8 ± 0,616**	**21,85 ± 0,657**	**17,35 ± 2,231**	**18,1 ± 2,189**
$Paco_2$	**5,11 ± 0,634**	**5,517 ± 0,117**	**5,035 ± 0,885**	**5,373 ± 0,292**

analysis of variance. To eliminate initial sample number differences per trial, the stages were expressed as percentages of the initial total number of embryos used for each weekly trial.

Results

The analysis of variance between embryo growth in Earle's medium and amniotic fluid is shown in Table I. There was no significant difference for the degenerate embryos, morulae and blastocysts A, B or C. However, there was a significant difference for stage D blastocysts. The overall blastulation rate to stage D for Earle's medium was 58,2%, whereas that for amniotic fluid was 78,6%. The change in osmolarity, pH and HCO_3^- and $PaCO_2$ content for the two fluids is shown in Table II. Amniotic fluid seemed less stable over the 3-day period than Earle's medium but the composition of both fluids after culture was not significantly different from their respective compositions before culture. However, amniotic fluid was more variable in composition than Earle's medium with regard to osmolarity and HCO_3^- content.

Discussion

The overall low blastulation rate compared with that reported by Gianaroli *et al.*,[4] was due to the acceptance of all embryos that were flushed; there was no selection of only normal two-cell embryos such as occurred in Gianaroli *et al.*'s trial. In our laboratory, where mouse embryos are used routinely for testing media, we have found that approximately 20% of all embryos obtained are degenerate or uncleaved when the above method of superovulation is used. If this is taken into consideration, then the corrected blastulation rate to stage D blastocyst for Earle's medium would be 66,14%, and for amniotic fluid 87,14%. The results indicate that amniotic fluid is a good culture medium for mouse embryos despite minor variations in osmolarity and HCO_3^- concentration. It remains to be shown whether it is necessary to adjust these parameters before using amniotic fluid for human IVF. The lack of statistical difference between embryos in the earlier stages of development may be due to the small numbers involved. However, the small difference at each stage accumulates and becomes evident in the stage D blastocysts, which have had to go through the earlier stages before reaching this stage. Amniotic fluid represents a readily available simple medium that is very effective in culturing early mouse embryos, and should be investigated further for application to human IVF.[4]

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