In vitro culture of mouse embryos in human amniotic fluid

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Summary

Human amniotic fluid was compared with Ham's F-10 culture medium as a possible alternative for use in *in vitro* fertilisation. The cleavage success of mouse embryos in human amniotic fluid (experimental group) was 92% compared with 86% in Ham's F-10 medium. It is concluded that human amniotic fluid is a viable alternative culture medium for mouse embryos.

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Most of the culture media that are commercially available and used in *in vitro* fertilisation (IVF) are expensive and, from a biological point of view, synthetic. Because human amniotic fluid is a physiological, balanced ultrafiltrate, it has been considered as an inexpensive alternative culture medium in IVF.

A study of the development of mouse embryos in human amniotic fluid was undertaken to assess the suitability of this as an optional culture medium in human IVF.

Methods

Sterile human amniotic fluid was obtained from patients 16-20 weeks pregnant and undergoing routine amniocentesis for early diagnosis of fetal abnormality. The amniotic fluid was centrifuged at 2 500 rpm for 10 minutes to remove the cellular component. The supernatant was then heat-inactivated at 56°C for 30 minutes and antibiotics (0,1 ml of a penicillin solution 10 000 μ/ml and a streptomycin sulphate solution 10 000 μg/ml) were added. The fluid was filtered (0,22 μm filter unit; Millex-GS, Millipore Corp., Bedford, Mass., USA) and stored at 4°C in sterile tissue culture tubes (Falcon 3001, Dickinson & Co., Oxnard, Calif., USA) for 7 days. Twenty-four hours before use, the amniotic fluid was equilibrated at 37°C under 5% CO_2 in air. Preparation of Ham's F-10 medium (Flow-Bios Laboratories, Johannesburg) has been described previously.[1]

Immature female C57 BL F1-generation mice were super-stimulated with 10 IU pregnant mare serum gonadotrophin (PMSG, Folligon, Intervet, Kempton Park, Tvl) given intramuscularly. Forty-eight hours later 10 IU human chorionic gonadotrophin (HCG, Propan Ethicals, Sandton, Tvl) were injected intramuscularly. The oviducts were flushed with phosphate-buffered saline 36 hours after the mice had mated and 656 2 - 4-cell embryos were obtained. Only normal embryos were used in the experiment.

The mouse embryos were randomly assigned to be cultured in either 3 ml Ham's F-10 culture medium supplemented with 15% fetal calf serum or 3 ml of human amniotic fluid. The embryos in the different culture media were incubated in sterile tissue culture tubes (Falcon 2001F, Dickinson & Co.) under a constant atmosphere of 5% CO_2 in air at 37°C for 72 hours. The development of the embryos was morphologically assessed using a Nikon SMZ 10 stereoscopic microscope.

Results

Of the 656 2 - 4-cell mouse embryos only 621 were normal after flushing and thus used in this study. A control group of 322 mouse embryos was cultured in Ham's F-10 medium and 299 embryos were cultured in human amniotic fluid. The cleavage rate for embryos cultured in Ham's F-10 medium was 86% compared with 92% for those cultured in amniotic fluid (Table I).

Discussion

Human amniotic fluid is an ultrafiltrate produced *in vivo* and less variable in chemical composition for a specific gestational period than serum, plasma and the more complex media that are commercially available.[2-5]

The consistency of human amniotic fluid, even between donors at the same stage of pregnancy, makes it a stable

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TABLE I. CLEAVAGE OF MOUSE EMBRYOS IN HAM'S F-10 MEDIUM AND HUMAN AMNIOTIC FLUID

	No. of mouse embryos			Degenerate		Mouse embryos after 72 h: Morulae		Mouse embryos after 72 h: Blastocysts	
Culture medium	2-cell	3-cell	4-cell	No.	%	No.	%	No.	%
Ham's F-10 + 15% serum	**184**	**60**	**78**	**8**	**2,5**	**37**	**11,5**	**277**	**86,0**
Human amniotic fluid	**211**	**51**	**37**	**7**	**2,3**	**17**	**5,7**	**275**	**92,0**

alternative culture medium. It also contains known and possibly unknown growth factors which could enhance embryo development.[2]

Our results indicate that mouse embryo development in human amniotic fluid is as good as that obtained in Ham's F-10 medium. It is thus possible that human amniotic fluid could be used for human IVF, as has been reported by Gianaroli *et al.*[2]

The cost-effectiveness of this alternative culture medium could be significant in a human IVF programme and should be investigated.

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