In vitro culture of mouse embryos in human amniotic fluid


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.

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 µg/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% CO₂ in air. Preparation of Ham's F-10 medium (Flow-Bios Laboratories, Johannesburg) has been described previously.

Immature female C57 BL F1-generation mice were super-stimulated with 10 IU pregnant mare serum gonadotrophin (PMSG, Folligon, Intervet, Kempton Park, Tvl) given intra-muscularly. 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% CO₂ 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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<th>TABLE I. CLEAVAGE OF MOUSE EMBRYOS IN HAM'S F-10 MEDIUM AND HUMAN AMNIOTIC FLUID</th>
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<td>Culture medium</td>
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<td>Ham's F-10 + 15% serum</td>
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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.

REFERENCES

Mouse embryos cultured in amniotic fluid

E. E. OETTLÉ, K. WISWEDEL

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 HCO3 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.

Andrology Laboratory, Department of Obstetrics and Gynaecology, University of Cape Town and Groote Schuur Hospital, Cape Town

E. E. OETTLÉ, B.V. Sc.

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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.1,2 Much of the quality control is outside the scope of the IVF laboratory, for example the source of original chemicals and conditions during distribution.

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.2 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 (PaCO2) and HCO3 (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