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In vitro application of Matrigel enhances human blastocyst formation and hatching

Marefat Ghaffari Novin[1] M.D., Ph.D., Mahnaz Heidari[2] M.Sc., Mahdi A Akhondi[1] Ph.D., Mahmood Jeddi Tehrani[3] Ph.D.

1 Reproductive Biotechnology Research Centre, Avesina Research Institute, ACECR, Tehran, Iran.
2 Nanobiotechnology Research Centre, Avesina Research Institute, ACECR, Tehran, Iran.
3 Monoclonal Antibody Research Center, Avesina Research Institute, ACECR, Tehran, Iran.

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Abstract:

Background: Matrigel (extracellular matrix) can improve the growth of many cell types *in vitro*.

Objective: The aim of the present study was to determine the effect of Matrigel on the development of 2-4 cells human embryos in culture.

Material and Methods: Surplus 2-4 cells human embryos, resulting from ICSI, were divided into two groups (control and test). Quality of embryos in both groups was morphologically similar. The test group (n=140) was cultured in Hams' F10 supplemented with 10% human serum albumin and 150 µl liquid Matrigel. The control group (n=140) was cultured in the same medium devoid of Matrigel. Embryos were cultured for an additional 4 days and their morphology was assessed every 24 hours. Both groups were then statistically compared.

Results: The percentage of the human embryos that reached the morula stage in the control and test groups were 79.2% and 80%, respectively ($p>0.05$). However, 36.4% of embryos reached the blastocyst stage in the test group as compared to 5.7% in the control group after 144 hours in culture. This difference was statically significant ($p<0.01$). In addition, culture of embryos on Matrigel and medium versus medium alone significantly improved in vitro hatching (25.7% versus 3.5%; $p<0.01$).

Conclusion: Matrigel at low concentration enhances human blastocyst formation and hatching *in vitro*.

Key words: *Matrigel, Extracellular matrix, Human embryos, Blastocyst, Hatching.*

Introduction

Although the use of assisted reproductive techniques (ART) has rapidly expanded, the pregnancy rate has not been impressive at approximately 20% in non-selective cases (1).

One of the primary reasons for low implantation rate has been the continued practice of transferring embryos to the uterus on day 2 or day 3 at the cleavage stage. This early transfer is required due to the reduced cleavage rate and poor viability of human embryos during prolonged culture. Indeed, many human embryos display a 6-cell block in vitro; only 25-30% will form blastocysts using standard culture condition (2,3). In addition, 2 to 4 cell stage embryo transfer is too early to allow perfect synchronization between the embryo and the uterus. This, together with poor embryo quality, both morphologic and metabolic, lowers the implantation rate.

To overcome these problems, it must be focused on improving the quality of the transferred embryos and/or the synchrony between embryo transfer and implantation. The blastocyst culture provides greater opportunities for selection of more viable and genetically normal embryos (4,5). This approach also provides more physiological

Correspondence Author:
Dr Marefat Ghaffari Novin, Reproductive Biotechnology Research Center, Avesina Research Institute, Tehran , Iran.
E-mail: mghaffarin@yahoo.com

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synchronization of the embryo stage with the endometrium (4). Therefore, the transfer of human blastocysts in ART should result in an increased implantation rate and enable the transfer of fewer, but higher quality of embryos.

Numerous culture systems with or without co-culture with helper cells can successfully support the development of human embryos into the blastocyst stage (6,7). However, there is not a standard protocol for blastocyst culture in practice due to some reasons. For example, the potential negatives of co-culture during human IVF are exposure of embryos to animal cells/proteins and the possibility of disease transmission. In addition, the co-culture technique is fairly labour intensive and requires tissue culture expertise, and the use of dividing cells can potentially introduce considerable variability into the embryo culture system (8).

Data from several investigations (9,10) indicate that cultivation of embryos on basement membrane protein coated substrates can enhance aspects of early mouse embryonic development. Matrigel is a commercially available basement membrane preparation extracted from the mouse Engelbreth-Holm-Swarm tumour whose components and their relative proportions are typical of those present in many basement membranes (11). Its major component is laminin, followed by collagen IV, heparin sulfate proteoglycans, entactin, and nidogen. It contains growth factor (12) and is known as a survival factor (13). ECM improves the growth of many cell types in culture such as human epithelial endometrial cells (14). The effect of Matrigel on animal embryonic development has been studied (9,15-17). However, little is known about its effect on human embryonic development. In this study, the potential use of Matrigel (ECM) was investigated as an alternative culture system for human embryo development.

Materials and methods

This study was an experimental prospective study on surplus 2-4 cells embryos of infertile couples, who attended to Avesina Infertility Clinic for ART cycle, and did not want to freeze their embryos. All surplus embryos were used with informed patient consent and Ethical committee approval.

In all patients, aged 20-40 years, gonadotrophin-releasing hormone (GnRH) analogue, buserelin (Suprecur; Hoechst, Frankfurt, Germany) was administered to achieve pituitary down-regulation from the preceding mid-luteal phase in a long stimulation protocol. Controlled ovarian stimulation was carried out with HMG (Merional, Switzerland). When the mean diameter of the leading follicle was >18 mm and there were at least three follicles with a mean diameter of ≥16 mm, 10,000 IU of Human chorionic gonadotrophin (HCG, Pregnyl; Organon) was administered to induce the final stage of oocyte maturation. Oocyte retrieval was performed by transvaginal ultrasound-guided aspiration under i.v. anaesthesia 36 hours after HCG administration.

Embryo culture

Oocytes were inseminated by ICSI, 3-4 hours after oocyte retrieval and were then cultured in Hams' F10 supplemented with 10% human serum albumin under mineral oil in a humidified incubator with 5% CO_2 and 95% air at 37 °C for two days. The embryos were then evaluated using embryo scoring (18) and around three good quality embryos were selected for embryo transfer. Surplus 2-4 cells human embryos were randomly divided into two groups (control and test). The medium was changed only at this time. Quality of embryos in both groups was morphologically similar.

Type HA millicell inserts pore size 0.5µm (Millipore, UK) were placed into wells of a Sterilin 24 (1 cm) well culture plate. Approximately 150 µl of Matrigel (Uniscience, Collaborative Biomedical products, Bedford, USA) in gel form was layered onto the insert, ensuring the bottom was completely covered. In the test group, embryos (n=140) were cultured in Hams' F10 supplemented with 10% human serum albumin and Matrigel. In control group, embryos (n=140) were cultured in the same medium devoid of Matrigel. The culture plate was then placed in an incubator gassed with 5% CO_2 at 37° C for an additional 4 days.

Embryo evaluation

The development of embryo was assessed every 24 hours to determine the number of embryos which reached the morula stage after 3 days (72 hr) in culture and the number which reached the blastocyst stage after 5 days (120 hr) in culture (18,19). Hatching was assessed after 5 days (120 hr) and 6 days (144 hr) in culture. Full expansion of the blastocyst was recorded when the central cavity was at least two thirds of the whole blastocyst volume, and visibility of the inner cell mass was checked. Embryo degeneration was recorded when single blastomeres could not be clearly distinguished, the cytoplasm was dark, embryo fragmentation was high and no definite

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stage of development could be recognized (19).

Statistical analysis

Differences in the rates of morula, blastocyst formation and hatching between test and control groups were analysed by X^2 test. Values of $p < 0.05$ were reported as being statistically significant.

Results

Table I compares the percentage of 2-4cells human embryos reaching the morula, blastocyst and hatching stages of development in the control and experimental groups. The percentage of the human embryos that reached the morula stage in the control and experimental groups were 79.2% and 80%, respectively. This difference was not statistically significant ($p>0.05$). However, 36.4% of embryos reached the blastocyst stage in the experimental group as compared to 5.7% in the control group after 5 days in culture. This difference was statically significant ($p <0.01$). In addition, when these embryos were evaluated 24 hours later, the stimulatory effect of Matrigel on development through to blastocyst hatching was even more obvious. Indeed, 3.5% of medium-cultured embryos had hatched compared with 25.7% of medium and Matrigel- cultured embryos ($p <0.01$). Even morphology was improved in the media enriched with Matrigel, with larger blastoceles and more visible ICM.

Table I. The effect of Hams F10 with Matrigel on the embryo development compared with the same medium devoid of Matrigel.

	No. of embryos reaching the indicated stage/ total no. of embryos cultured		
Medium	8-16 cells	Blastocyst	Hatching
Hams F10	111/140	8/140	5/140
	(79.2%)	(5.7%)	(3.5%)
Hams F10	112/140	51/140	36/140
+Matrigel	(80%)	(36.4%)	(25.7%)
P value	NS	<0.01	<0.01

Note: NS= not significant

Discussion

The modulation of cellular function and development both in vivo and in vitro is a function of soluble signals such as those derived from growth factors, cytokines and hormones, and insoluble signals arising from the ECM. The present study shows that Matrigel enhances human blastocyst formation and hatching in vitro. In agreement of our study, it has been shown that blastocyst development on fibronectin coated dishes is significantly greater than this in the control group (10). In other study, an extracellular matrix product isolated from human placenta was used for embryo development (15) and was observed positive effect of ECM on blastocyst formation.

The positive effects of Matrigel on embryo development are probably due to its composition. Matrigel contains the same structural proteins and growth factors as are found in the endometrial matrix, such as laminin, collagen IV, heparin sulfate proteoglycans, entactin, nidogen, growth factors, and tissue plasminogen activator (11). These components in the culture medium may recreate an environment similar to the uterine milieu, and therefore may stimulate not only embryo survival and growth, but also its differentiation and the expression of membrane receptors and the secretion of specific growth factors.

Matrigel has been shown to contain variable amounts of epidermal growth factor (EGF) (0.5-1.3 ng/ml), platelet-derived growth factor (PDGF) (5-48 pg/ml), insulin-like growth factor-I (IGF-I) (11-24 ng/ml) and transforming growth factor-β (TGF β) (1.7-4.7 ng/ml) (20). All of these identified factors can influence specific aspects of embryonic development (21, 22).

The present study also shows that Matrigel improve hatching of embryo in vitro. Lazzaroni et al (1999) showed that the use of Matrigel enhances hatching of mouse embryo in vitro (16). Protease activity in the early embryo can also be affected by culture substrata and ECM components. The type of ECM was shown directly to influence embryonic secretion of basemembrane degrading proteases, such as urokinase plasminogen activator (23). Higher collagenase type IV activity was seen when trophoblasts were cultured with laminin versus on plastic substrata (24). Laminin also promoted in vitro hatching of cultured human embryos (25). Therefore, all of these data suggest that Matrigel components (ECM) could increase hatching process by increasing proteases secretion from embryo.

Conclusion

Matrigel enhances human blastocyst formation and hatching in vitro. The combination of blastocyst culture media with growth factor and ECM components may prove to be a new avenue

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for formulation of more physiological culture systems.

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Editorial Comment

I have read with interest the work of Ghaffari Novin and his associates. The application of Matrigel on animal embryonic development has been studied previously (1,2). However, the effect of Matrigel on in vitro culture of human embryos has not been reported before. The results from

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Ghaffari Novin's study showed that application of ham's F 10 supplemented with HSA+ Matrigel significantly increased the surplus human embryos reaching blastocyst stage. It is, however, important to note that several studies suggest that optimal conditions for the culture of human embryos involve the use of sequential medium with respect to not only energy substrates, but also other components such as amino acids and macromolecules. Although, Ham's F10 is used in embryology laboratories, but mainly for the use of early embryo development. The application of sequential microdrop system is recommended for embryo development to blastocyst stage (3).

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Dr. Mohammad Ali Khalili
Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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