ANIMAL EXPERIMENTATION

Vero Cells, But Not Oviductal Cells, Increase the Hatching Frequency and Total Cell Count of Mouse Blastocysts Partly by Changing Energy Substrate Concentrations in Culture Medium

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Purpose: To investigate the embryotrophic mechanisms of Vero and oviductal cells coculture.

Methods: Mouse embryos were cultured in Chatot, Ziomek, and Bavister medium (CZB), in modified CZB media (MM) with nutrient concentrations adjusted to that found in conditioned media after different periods of Vero cells or oviductal cells culture, in reconstituted medium (RM) containing the purified >100-kDa components of Vero cell conditioned medium that had been reconstituted with CZB medium, and cocultured with Vero cells with an interposing membrane.

Results: The blastulation rate was not different among embryos cultured in different Vero-cell-derived MMs. Nine-hour Vero-cell-derived MM significantly increased the total cell number and hatching frequency of the embryos. There was no difference in these parameters with oviductal-cell-derived MMs. The RM of Vero cells did not possess embryotrophic activity. The presence of a porous membrane between Vero cells and embryos did not affect the embryotrophic activity of coculture.

Conclusions: Vero cells, but not oviductal cells, improved mouse embryo development partly by modifying the energy substrate concentration in culture medium.

KEY WORDS: coculture; embryo; glutamine; lactate; pyruvate.

INTRODUCTION

Coculture is one of the methods to improve embryo development in vitro. This technique has been used successfully with human embryos (1,2). Despite its success, coculture has not been the main method for embryo culture in human in vitro fertilization (IVF) program because of the complexity in its implementation as a routine service. Therefore, the recent development of sequential culture, the use of different culture media for culturing embryos at different stages of development, has rapidly become the method of choice for improving the outcome of human IVF (3). However, these two methods are not mutually exclusive, and in fact they can complement each another.

Our unpublished data show that more mouse blastocyst hatch after human oviductal cell coculture in G1.2 and G2.2, one of the commonly used commercial sequential culture media, than those cultured in the sequential media alone. Granulocyte-macrophage colony-stimulating factor, a cytokine produced by the human fallopian tube (4), has also been shown to improve human embryo development in the same sequential culture system (5). Oviductal cell is one

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of the somatic cells commonly used in coculture. These data suggest that the present sequential culture system can be further improved by incorporating coculture. To eliminate the complexity of coculture and the possible transmission of infectious disease from the somatic cells during coculture, studies on the mechanism of embryotrophic activity in coculture are necessary. This will enable us to provide an environment similar to coculture without the use of somatic cells.

It is known that the embryotrophic effect of coculture is not species-specific. We have established a mouse model to study the mechanisms of coculture. In this model, a specific strain of mouse with low developmental potential in most commonly used mouse culture media (CZB, KSOM, mTF) is used. As coculture is known to be embryotrophic, the use of this mouse strain will allow much room for improved development induced by changes in culture condition. On the other hand, the sensitivity of detecting such an improvement will be lower if mouse strains with high development potential in standard culture conditions are used as the treatment-induced changes in development in these mice would not be noticeable easily. Presumably, this is one of the reasons why progress in studies on the mechanism of coculture is slow. With the present model, we have been able to detect the presence of three embryotrophic factors from human oviductal cell conditioned medium (6).

Vero cells derived from African Green Monkey kidney are the commonest somatic cell used for coculture with human embryos. Vero cell coculture improves the development of mouse embryos in vitro (7–11). The mechanism(s) by which Vero cells improve embryo development are not fully understood. Various reports show that the concentrations of energy substrates, including some amino acids such as glutamine, affect the development of preimplantation embryos (9,12–15). During coculture, the embryos compete with the helper cells for energy substrates. The first objective of this study is to investigate the effect of modification of energy substrate concentration in the culture medium by the Vero cells during coculture on mouse embryo development. The observed effect was compared to that of a human oviductal cell coculture system.

The somatic cells in coculture may produce embryotrophic factors (1,8,11,16) that stimulate embryo development in vitro. We have recently partially purified three high molecular weight embryotrophic factors

(>100 kDa) from human oviductal cells (6,17). The identities of these factors are unknown. We currently named these factors as Embryotrophic Factor-1 (ETF-1), ETF-2, and ETF-3. The second objective of this report is to determine whether Vero cells produce similar high molecular weight embryotrophic factors. The final objective of this study is to determine if cell-to-cell contact is necessary for the observed embryotrophic activity of Vero cells.

MATERIALS AND METHODS

Culture of Vero Cells and Oviductal Cells and Collection of Conditioned Medium (CM)

Human oviductal cells were prepared as previously described (18). In brief, human fallopian tube tissue was obtained from patients admitted for tubal ligation or hysterectomy due to uterine fibromyoma. Informed consents were obtained from these patients for donating their fallopian tubes for this study. The University Ethics Committee, The University of Hong Kong, approved the study protocol. Vero cells were a kind gift from Dr. D. Sakkas, University of Birmingham, Birmingham, U.K. Oviductal cells and Vero cells were cultured in Dulbecco's minimal essential medium/Ham's F12 medium (1:1 v/v; DMEM/F12) supplemented with human preovulatory serum (sDMEM/F12). These cells were cryopreserved in liquid nitrogen before use. Frozen oviductal cells were used because of the logistic reason that donated oviductal tissue was not always available. Our data showed that the frozen oviductal cells still produced human oviductal specific glycoprotein (YL Lee, CKF Lee, WSB Yeung, unpublished data) and stimulated human and mouse embryo development (2,6,17) after thawing.

Prior to experimentation, frozen oviductal cells and Vero cells were thawed in a water bath at 37°C and rinsed thrice in PBS. Oviductal cells were seeded at a density of 5×10^4 cells/well and cultured for 48 h whereas Vero cells were seeded at 6×10^5 cells/well and cultured for 24 h in a 24-well culture plate before collection of conditioned medium (CM). Preliminary experiment showed that under these conditions, the somatic cells possessed the maximal embryotrophic activity. The cells were then washed with Chatot, Ziomek, and Bavister medium (CZB; 19). The medium contained 81.62 mM NaCl, 4.83 mM KCl, 1.18 mM KH_2PO_4 , 1.18 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 25.12 mM NaHCO_3 , 1.70 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$,

31.30 mM sodium lactate, 0.27 mM sodium pyruvate, 0.11 mM EDTA, 1 mM glutamine, 5 mg/mL bovine serum albumin (BSA), 100 IU/mL sodium penicillin, and 0.7 mM streptomycin. The spent media (CM) after culturing these cells for 0, 3, 6, 9, and 24 h in CZB were collected. These media were labelled as 0CM, 3CM, 6CM, 9CM, and 24CM respectively.

Mouse Embryo Culture

Mature MF1 female mice (6–8 weeks old) were superovulated with 5 i.u. pregnant mare's serum gonadotrophin (Sigma, USA) followed by an injection of 5 i.u. human chorionic gonadotrophin (hCG; Sigma, USA) 46 h later. MF1 females were then mated with proven fertile BALB/c males. Our data showed that this mouse strain had a 2-cell block when cultured in mouse tubal fluid medium (mTF), and the development of the embryos in CZB was similar to those cultured in high potassium simplex optimized medium (KSOM), another commonly used mouse embryo culture medium.

The day of the presence of vaginal plug was considered as Day 1. The zygotes were recovered 24 h post-hCG from the oviductal ampullae into Hepes-buffered M2 medium containing 0.8 mg/mL hyaluronidase (Sigma, USA). The zygotes were washed thrice in 250 μ L M2, followed by one wash in CZB before being cultured in different media according to the experimental design (see later) for the first 48 h at 37°C in 5% CO₂ in air. Our unpublished data showed that embryos cultured in CZB without glucose for the first 48 h had better development than those cultured in M16 without glucose. Embryos were subsequently cultured in glucose-containing M16 medium (20) for two reasons. First, glucose is required for blastocyst development (21). Second, the use of EDTA in CZB after compaction might reduce blastocyst development and total cell number in mouse (22). Furthermore, the blastulation rates of embryo cultured in CZB/M16 and CZB/CZB + glucose are similar.

Determination of Substrate Concentrations in CM

Lactate and Pyruvate. The lactate and pyruvate concentration levels in the CMs were determined by Sigma diagnostics kits (Sigma, USA) with procedure number 826-UV and 726-UV respectively. The assays

were based on the following reversible reaction of lactate dehydrogenase.

To measure lactate, the reaction was carried out in excess NAD. The pyruvate formed was immediately reacted with hydrazine in the reaction mixture. The concentration of lactate in the CMs was proportional to the change in absorbance at 340 nm as a result of NADH formation.

The pyruvate was determined in the presence of excess NADH. In this condition, essentially all the pyruvate was converted to lactate. The reduction in absorbance at 340 nm due to oxidation of NADH to NAD was proportional to the amount of pyruvate present in the CMs.

Glutamine. The concentration of glutamine in the CMs was evaluated by a Beckman high performance capillary electrophoresis P/ACE System 5010 (Beckman Instruments Inc., USA). Uncoated capillary (75 μ m $\times$ 47 cm) containing phosphate buffer (pH 7.8) at 25°C was used to separate glutamine from other components in the CMs. Constant voltage of 10 kV was applied during electrophoresis. The running polarity was from negative to positive. Forty-five microliters of CM was mixed with 5 μ L of 2 mM arginine. Arginine was chosen as an internal standard during capillary electrophoresis because it was well separated from all the other known amino acids in the electropherogram. Our preliminary experiments also showed that Vero cells and oviductal cells produced no detectable arginine peak in the electropherogram. The amino acids were analysed by UV detection at 200 nm. The peak area of glutamine relative to arginine was used to quantify the amount of glutamine in the CMs.

Preparation of the Modified Media (MM)

After the determination of energy substrate concentrations in the CMs, modified CZB media (MM), containing the three substrates at concentrations equivalent to their levels in oviductal and Vero cell CMs at each time point were prepared. These media were labeled as 0MM, 3MM, 6MM, 9MM, and 24MM, which corresponded to the CM after 0-, 3-, 6-, 9-, and 24 h of conditioning respectively. One-cell mouse embryos were cultured in 20 μ L MMs for 48 h

and subsequently in M16 medium for the rest of the culture.

Determination of the Reconstituted Media (RMs) Effect on Embryo Development

The high molecular weight fraction of the CMs from Vero cell culture was isolated by ultrafiltration of 0CM, 3CM, 6CM, 9CM, and 24CM through a CentriconTM 100 (Amicon, Beverly, MA, USA) with molecular weight cutoff of 100 kDa. The high molecular weight fraction was then reconstituted in CZB with BSA to the original volume to form the reconstituted medium (RM). The RMs were labelled as 0RM, 3RM, 6RM, 9RM, and 24RM respectively. Thus RM contained the high molecular fraction of CM but the same energy substrate levels as fresh CZB medium. One-cell embryos were cultured in 20- μ L RM droplets for the first 48 h. The embryos were then transferred to M16 medium for the subsequent culture.

Effect of Cell Contact on Embryo Development

A microporous membrane insert (Nunc, Roskilde, Denmark) was first incubated in CZB medium for 24 h before use. The membrane was uncoated and had a pore size of 0.02 μ m. It was placed between the mouse embryo and the Vero cells. The embryos were cultured on top of the insert whilst the coculture cells were on the bottom of the culture dish. The development of these embryos was compared to those cultured in CZB alone and to those cocultured with Vero cells but without the insert.

Assessment Parameters

Based on the presence of two pronuclei, the fertilization rate of this mouse strain ($\varnothing$ MF1 $\times$ δ BALB/c) was only around 50–60%. This was consistent with

our unpublished data that the percentage of 2-cell embryos after culturing in CZB, KSOM, mTF, and G1.2/G2.2 ranged from 54 to 66%. The number of embryos reaching fully expanded and hatching blastocyst stage were recorded at approximately 120 and 144 h post-hCG respectively. These numbers were normalized against the number of 2-cell embryos formed with the corresponding experimental group. The cell count of blastocysts was determined at 120 h post-hCG with Giemsa stain, according to the method of Tarkowski (23).

Data Analysis

Each experiment was repeated at least thrice. About 15–20 2-cell embryos were used for every treatment group in each replicate experiment. Chi-square analysis was used to compare the rate of blastulation and hatching of embryos. Student's *t* test was used to compare the total cell count per blastocyst (TCN). A *p* value of <0.05 was considered as significant difference.

RESULTS

Effect of Substrate Modification on Embryo Development

CZB is a glucose-free and serum-free medium. It reportedly contains 31.3 mM lactate, 0.27 mM pyruvate, and 1 mM glutamine (19). Their concentrations as determined by the biochemical methods described here were 30.9 ± 0.62 mM, 0.263 ± 0.01 mM, and 1.00 ± 0.01 mM respectively (Table I). After Vero or oviductal cell culture, the lactate concentration in CZB decreased slowly; its level at 24 h for both cell types was significantly lower than that in the fresh CZB. Both cell types utilized more pyruvate

Table I. Concentration of Lactate, Pyruvate, and Glutamine in CZB-Conditioned Media After Culturing Vero and Oviductal Cells for 0, 3, 6, 9, and 24 h (*n* = 3)

CM	Lactate (mM)		Pyruvate (mM)		Glutamine (mM)	
	Vero	Oviduct	Vero	Oviduct	Vero	Oviduct
0	30.9 ± 0.62		0.263 ± 0.01		1.00 ± 0.01	
3	29.9 ± 0.88	28.4 ± 1.28	0.223 ± 0.001^a	0.241 ± 0.006^a	0.92 ± 0.01^a	$0.71 \pm 0.03^{a,b}$
6	28.9 ± 0.39	27.2 ± 1.94	0.220 ± 0.01	0.209 ± 0.007^a	0.84 ± 0.02^a	$0.69 \pm 0.01^{a,b}$
9	27.7 ± 1.07	26.6 ± 2.41	0.192 ± 0.02^a	0.192 ± 0.007^a	0.81 ± 0.02^a	$0.56 \pm 0.04^{a,b}$
24	27.7 ± 0.33^a	27.1 ± 0.64^a	0.176 ± 0.005^a	0.166 ± 0.004^a	0.71 ± 0.01^a	$0.45 \pm 0.07^{a,b}$

Note. Values are mean $\pm$ SEM.

^a Significantly different (*P* < 0.05) from 0CM within the same column.

^b *p* < 0.05 between the two cell types for the same nutrient within same duration of conditioning.

and glutamine than lactate. The levels of these two substrates were 69 and 70% of the starting concentrations for Vero cells, and 64 and 46% for oviductal cells after 24 h of culture. These concentrations were significantly different from that in CZB before cell culture.

The results of using MMs to culture mouse embryos are shown in Fig. 1. The values in the Figure represent the combined results of five replicate experiments. Compared with CZB (0MM), there was no difference in blastulation rate when embryos were cultured in Vero-cell-derived MMs at all the time points measured. However, embryos grown in the 9MM derived from Vero cell culture had significantly higher TCN (72 ± 4.8 ; $P < 0.01$) and hatching frequency (14%; $P < 0.05$) than the control embryos cultured in 0MM. The corresponding control values were 49 ± 3.2 and 7%. When embryos were cultured in media modified according to the CMs derived from oviductal cell culture, they were not different from the control embryos in any parameter measured at any time point.

Effect of RMs on Embryo Development

The effects of RMs from Vero cell's conditioned medium on embryo development are shown in Table II. No significant difference was found among all the groups in terms of blastulation rate, TCN, and hatching frequency.

Effect of Cell Contact on Embryo Development

The effects of coculture with or without cell contact on embryo development are shown in Table III. At 120 h post-hCG, the rate of blastocyst formation was 32% when embryos were cultured directly on the Vero cell monolayer. The blastulation rate did not change when a porous membrane was interposed between the embryos and the Vero cells (32%). These blastulation rates were significantly higher than that

Fig. 1. Blastulation rate, hatching frequency, and total cell count per blastocyst of mouse embryos cultured in energy-substrate-modified media (* $P < 0.05$). Number in each bar represents total number of 2-cell embryos used in that treatment group.

of the control (19%). The TCN was also higher in the coculture groups than that of medium alone culture, though the difference had not yet reached statistical significance.

Table II. Effects of Reconstituted Media (RM) on the Development of Mouse Embryos In Vitro

	Blastulation rate			Hatching frequency		
	No. of 2-cell embryo	Blastocyst n (%)	Blastomere count	No. of 2-cell embryo	>Hatching n (%)	Hatched n (%)
Control	149	49 (33)	51 ± 3.1	106	18 (17)	7 (7)
RM3	160	56 (35)	51 ± 2.7	122	24 (20)	9 (7)
RM6	149	53 (36)	54 ± 2.5	127	24 (19)	9 (7)
RM9	154	54 (35)	51 ± 2.2	105	18 (17)	11 (10)
RM24	176	51 (29)	58 ± 2.9	125	19 (15)	10 (8)

Note. No significant difference was found among all the groups; >Hatching: number of embryos underwent or completed the hatching process; Hatched: number of embryo completed the hatching process. RM3, RM6, RM9, RM24: Media containing the high molecular weight fraction of the spent media that had been cultured Vero cells for the number of hour shown in the abbreviations.

Table III. Cell Contact Effect on the Development of Mouse Embryos In Vitro

	No. of 2-cell embryo	Blastocyst <i>n</i> (%)	Blastomere count
Control	118	34 (29)	48 ± 2.9
Vero cells without insert	103	59* (57)	52 ± 2.4
Vero cells with insert	119	59* (50)	54 ± 2.3

**p* < 0.05 (when compared with the control).

DISCUSSION

Previous studies (7–11) showed that Vero cells stimulate the blastulation rate of mouse embryos. The present report confirms these findings and further investigates the possible mechanisms of embryotrophic activity in this coculture system.

During coculture, the mammalian embryos compete with the helper cells for energy substrates, the concentrations of which are known to affect embryo development in vitro (13). In this study, Vero cells and oviductal cells consumed energy substrates at different rates. This difference in the consumption of energy substrates correlates with the ability of the MMs to support embryo development.

Many somatic cells are able to metabolize glutamine in the absence of glucose and even prefer this amino acid (24). Glutamine is a major source of energy, carbon, and nitrogen for mammalian cell growth (24,25). It is metabolized via glutamate and α -ketoglutarate before entering the tricarboxylic acid cycle. In this study, both the oviductal cells and the Vero cells used significant amount of glutamine in the glucose-free CZB medium. This is in agreement with the suggestion that the energy requirement of mammalian cells is met by aerobic oxidation of glutamine when the glucose concentration is low (25).

Although oviductal cells were seeded at a lower density than that of the Vero cells, the former utilized more glutamine than the latter. The higher utilization rate of glutamine by the oviductal cells suggest that either the cells are more actively dividing or they are using glutamine for other purposes e.g. protein synthesis. In this connection, human oviductal cells secrete high molecular weight embryotrophic factors in CZB (6,17) whereas similar embryotrophic factors cannot be identified in CZB after Vero cell culture in this study.

There is no difference in the consumption of lactate and pyruvate between the oviductal cells and Vero cells within the same culture duration, though the rate of utilization per cell is higher in the former

than in the latter. Both cell types utilize pyruvate in culture, but the rate of consumption is lower than that of glutamine. The lactate level in the culture medium only dropped slightly after oviductal and Vero cells culture.

When 1-cell mouse embryos were cultured in MMs derived from oviductal cell culture, there was no significant difference in embryo development among different MMs. This suggests that modification of nutrient levels do not contribute to the embryotrophic activity of human oviductal cells. In contrast, 9-h energy substrate levels in Vero cell cultures improve embryo development. Thus the embryotrophic activity of Vero cell coculture may operate partly via modification of the energy substrate levels. However, this embryotrophic activity of Vero cells is limited to a culture condition produced by the cells after 9 h of culture, as it is not seen in other MMs.

The concentrations of lactate and pyruvate in MMs derived from human oviductal and Vero cell types are similar. Therefore, the observed difference in embryotrophic activity of MMs derived from the two cell types is likely due to the difference in glutamine concentration. The lower glutamine concentration in oviductal MMs may lead to poorer embryo development. This is supported by observations both from this laboratory as well as from others (18). Our unpublished data (CK Fung and WSB Yeung) show that embryo development of the same mouse strain in CZB medium containing 0.5 mM glutamine is inferior to those cultured in the same medium containing 1 mM glutamine. Chatot *et al.* (19) replaced glucose by glutamine in CZB medium and significantly enhanced the development of random-bred 1-cell mouse embryos.

MM derived from 9-h Vero cell conditioned medium gives best support to embryo development in this study. The concentration of glutamine in this medium is 0.8 mM, which is slightly lower than the optimal glutamine concentration in CZB reported by Chatot *et al.* (26). Although they demonstrated that 1 mM glutamine in CZB was better than 0.5 and 0.1 mM in supporting 1-cell mouse embryo development (26), they did not study the effect of 0.8 mM glutamine. Moreover, only the concentration of glutamine was varied in their study, while that of the other nutrients remained unchanged. This differs from the present study when glutamine as well as pyruvate and lactate concentrations are altered in MMs. It is known that optimal embryo development in vitro can be obtained in different culture media containing the same components but at different concentrations (27). Thus

the beneficial effect of MM derived from 9-h conditioned medium is likely to result from the combined reduction in pyruvate, lactate, and glutamine.

The reported effects of glutamine on mouse preimplantation embryo development are conflicting (28). Different strains of mice may respond differently to glutamine treatment (29). Comparing with 0.5 mM and 2.0 mM glutamine, our unpublished data show that 1.0 mM glutamine increases TCN mainly by increasing the number of trophoctoderm cells. Incidentally, Devreker *et al.* (30) reported an increase in the number of trophoctoderm cells of human embryo after supplementation of the culture medium with 1 mM glutamine, though the increase was not yet large enough to reach statistical significance. Glutamine alone is as effective as a complete amino acid supplement in supporting the hatching of hamster blastocyst (31). The improved development of trophoctoderm may explain the effect of Vero cell derived MM on enhancing the hatching frequency of the treated embryos.

Edwards *et al.* (13) used a similar approach to demonstrate that bovine oviductal cells improved the suboptimal embryo culture conditions in TCM-199 by altering the carbohydrate concentrations in the medium. In their study, the authors determined the change in carbohydrate and amino acid concentrations in TCM-199 after oviductal cell culture, but the effects of the carbohydrate changes on embryo development was determined only in synthetic oviductal fluid medium and not in TCM-199-based medium. It is known that the uptake of one nutrient by mammalian cells is influenced by the concentration of other nutrients (32,33). Thus, the nutrient utilization rate by the oviductal cells in TCM-199 and synthetic oviductal fluid medium may not be the same. Moreover, there are complex interactions among energy substrates during embryo development *in vitro* (27). Any observed embryotrophic effect of MM is likely to be the consequence of changes in all the energy substrates together in the culture medium and not a result of changes in carbohydrate alone. Thus the results of this study had to be interpreted with caution.

Liu *et al.* (17) demonstrated secretion of embryotrophic factors by human oviductal cells. These embryotrophic factors have molecular weights of more than 100 kDa (6). RM has the same high molecular weight components as in each of the coculture systems but the same low molecular weight components as in the control CZB medium. The present study shows that RMs from Vero cells do not improve embryo development, suggesting that the embryotrophic activity

of the Vero cells reside in the fraction with molecular weight <100 kDa. Vero cells are known to secrete low molecular weight growth factors, e.g. leukemia inhibitory factor (16), growth factors (8), insulin-like growth factor binding proteins (11), cytokines (8), and embryotrophic peptides with molecular weight range between 6.5 kDa and 35.9 kDa (7).

When a microporous membrane that allowed free diffusion of molecules was placed between the mouse embryo and the Vero cell monolayer, no significant difference was found in the mouse embryonic development, suggesting that cell contact was not required for the embryotrophic activity of Vero cells. As the early embryo is enclosed within a noncellular zona pellucida, direct cell-to-cell contact between the blastomeres and other somatic cells is not possible even *in vivo*. Thus it is not surprising to find that this is not necessary in a coculture system. Similar observation was also found with the oviductal cells.

Our results showed that there was no difference in the blastulation rate in Vero cell MMs, whereas significant difference in blastulation rate could be found in the cell culture insert system. This may be due to the close vicinity of the embryos with the Vero cells in the coculture system and the observed embryotrophic effect is due to local changes in nutrient concentrations together with other low molecular weight embryotrophic factors. Coculture system is a static culture system. It allows the formation of concentration gradients of energy substrates around the embryos when the culture is left undisturbed. The concentration of the nutrients in the MM is the average of the nutrient concentration gradient in the CM. In other words, MM represents only the diluted effect of the modification in a coculture system.

The developmental outcome in this study appears low with blastulation rates ranged from 29–57%. These low blastulation rates are not due to the use of a suboptimal culture system (CZB/M16) because the blastulation rates for these embryos in another embryo culture medium, KSOM, is 35% (JS Xu, WSB Yeung, unpublished data), which is similar to the control group in the present report. The embryos have a 2-cell block in mTF and no blastocyst can be formed. Our data also show that the 1-cell embryo in this mouse strain is particularly sensitive to culture as the blastulation rate for 2-cell embryos obtained from the oviduct is over 90% in CZB/M16.

Despite the difficulty in culturing the 1-cell embryos of this mouse strain, they are used as our model for two reasons. First, because of their sensitivity to culturing condition, the environmental effect on development

can be detected relatively easily in terms of total cell number and blastulation rate of the resulting blastocyst. For many other mouse strains, more sophisticated endpoints, e.g. differential staining of inner cell mass and trophectoderm—trophoblast spreading area—have to be used, because vast majority of their embryos reach the blastocyst stage irrespective of the culture condition. Second, it is closer to the human assisted reproduction program in which embryos are cultured from the zygote stage. Although the result of this study may not be applied directly to human embryo culture, the approach of the study can be used to determine the appropriate energy substrate concentrations in the culture medium.

In summary, Vero cells, but not oviductal cells, may affect embryo development by modifying the concentration of nutrients in the culture medium. This beneficial effect of Vero cells was not via the production of high molecular weight embryotrophic factors and did not require cell-to-cell contact of Vero cells and the embryos. This effect of Vero cells is specific to a 9-h conditioning period. A recent paper also showed that human embryos are exposed to a dynamic change of growth factors, like IL-6, PDGF, and LIF during coculture with Vero cells (8). Although coculture effect is known not to be cell-type specific, the present data suggest that different cell types may exert their effects via different mechanisms.

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