Efficacy of Polyvinyl Alcohol Supporting the Development of Mouse Preimplantation Embryo In Vitro

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Abstract: The effect of polyvinyl alcohol (PVA) on the development of mouse preimplantation embryos was examined. In Hoppe and Pitts medium supplemented with 0.1 or 1.0 mg/ml PVA, a high percentage (comparable to BSA-supplemented medium) of mouse 1-cell embryos developed to the expanded blastocyst stage. However, in medium supplemented with 10.0 mg/ml PVA, no embryos developed to the blastocyst stage. These results indicate that, at optimum concentration, PVA supports embryo development from the 1-cell to the expanded blastocyst stage, and that the PVA-supplemented medium is a valuable chemically defined medium for mouse embryonic culture. Also, in this study, in the PVA-supplemented medium, percentages and speeds of embryo development from each stage (1-cell, 2-cell, 8-cell and early blastocyst) to the expanded blastocyst stage were almost the same as those in the BSA-supplemented medium. These results suggest that these embryos developed to expanded blastocysts via normal processes, and that PVA supports embryo development in each stage up to the expanded blastocyst stage. Incidence of partial hatching and complete hatching of blastocysts was clearly decreased in cultures of each embryo from 1-cell to the early blastocyst stage in the PVA-supplemented medium. It has been considered that protease may participate in the hatching process of blastocysts in vitro, thus, it is probable that the low hatching rate of blastocysts in the PVA-supplemented medium is due to a decline in protease synthesis and/or secretion.

Key words: Polyvinyl alcohol, Chemically defined medium, Mouse embryo, Hatching

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A number of media have been created for use in the development of mouse preimplantation embryos. Many different media have been found to support development of 1-cell embryos to blastocysts [1–7]. Many media used for development of such embryos are supplemented with bovine serum albumin (BSA) [1–9]. Although the mechanisms by which BSA supports embryo development have not been determined, a study has found that albumin is used by embryos as a source of amino acid, that it chelates heavy metals, and that trace contamination with BSA promotes development of embryos [9]. Incidence of development of early embryos and hatching of blastocysts correlates with the lot of BSA in medium [10, 11], therefore the effects of other supplements on embryo development may be masked in BSA-supplemented medium.

Mouse and hamster preimplantation embryos develop to blastocysts when the medium is supplemented with polyvinyl alcohol (PVA) [12, 13], however, hatching percentages of rabbit [14] and equine [15] blastocysts are decreased by PVA supplementation to the medium. Biggers *et al.* [13] demonstrated that 1-cell mouse embryos develop to blastocysts with normal cell numbers at a moderately reduced rate in PVA-supplemented medium, and that the percentages of partial hatching and complete hatching of blastocysts are markedly decreased in PVA-supplemented medium. Furthermore, the percentage of hatching of blastocysts is reduced when 8-cell stage embryos are cultured in PVA-supplemented medium [9]. It has been reported that the hatching rate of embryos in PVA-supplemented medium increases when essential and non-essential amino acids of Eagle medium are added [16]. However, the cause of the low hatching rates of mouse blastocysts in PVA-supplemented medium is not known.

For the purpose of creating a chemically defined

medium in which mouse preimplantation embryos can develop with high percentages, we examined the effect of PVA on embryo development, especially on hatching of blastocysts.

Materials and Methods

Animals

We used female (8~12 weeks old) and male (13~21 weeks old) ddY strain mice. Mice were maintained at 23°C, on a 14L:10D (light-on, 05:00) light-dark cycle.

Collection of embryos

Females were superovulated with intraperitoneal injections of 5 to 8 IU pregnant mare's serum gonadotropin (PMSG, Teikoku Hormone Mfg. Co., Tokyo, Japan), followed 48 h later by 5 to 8 IU human chorionic gonadotropin (hCG, Teikoku Hormone). Immediately after the hCG injection, the females were individually paired with males overnight. The females were checked the following morning for the presence of a vaginal plug. Females with a vaginal plug were sacrificed by cervical dislocation 18 to 20 h after hCG injection, and zygote-cumulus complexes were recovered from the ampullae. Cumulus cells were removed from the embryos by 0.1% hyaluronidase (H-6254, Sigma Chemical Co., St Louis, USA) treatment, and morphologically normal 1-cell stage embryos were selected.

Culture media

Hoppe and Pitts medium [17] supplemented with 50 μM EDTA was used (Table 1). Salts and organic reagents in the medium were purchased from Nacalai Tesque Inc. (Guaranteed Reagent, Kyoto, Japan), and potassium penicillin G and streptomycin sulphate were purchased from Meiji Seika Kaisha, Ltd. (Tokyo, Japan).

Embryo culture

To determine the suboptimum concentration of polyvinyl alcohol (PVA) in medium, 1-cell embryos were cultured in medium supplemented with 0.1, 1.0 or 10.0 mg/ml PVA (MW. 30,000~70,000. P-8136, Sigma) or 3 mg/ml BSA (A-6003, Sigma).

To investigate the efficacy with which PVA supports embryo development, 2-cell and 8-cell embryos and early blastocysts that had developed from 1-cell embryos in the BSA-supplemented medium were cultured in medium supplemented with 1.0 mg/ml PVA. Developmental potency of embryos was compared between the PVA-supplemented medium and the BSA-supplemented medium.

Table 1. Composition of Hopps and Pitts medium

Composition	Concentration	
	g/liter	molarity
NaCl	5.140	87.86
KCl	0.356	4.77
KH_2PO_4	0.162	1.19
$MgSO_4 \cdot 7H_2O$	0.294	1.19
$NaHCO_3$	1.900	22.62
Na-pyruvate	0.025	0.23
Na-lactate	2.417	21.57
Ca lactate $\cdot 5H_2O$	0.527	1.71
D-Glucose	1.000	5.56
K-penicillin-G	0.075	—
Streptomycin sulfate	0.050	—
Bovine serum albumin	3.0 (mg/ml)	—

Molarity values were calculated from g/liter data, except for BSA which is in mg/ml. Hoppe and Pitts (1973).

The developmental stage of embryos was checked every 6 h from the start of culture. Embryos were cultured according to the method of Brinster [18], with culture dishes kept in a CO_2 incubator (37°C, 5% CO_2 + 95% air).

Data analysis

Data obtained were analyzed by Chi-square test and Tukey's test [19] with $p<0.05$ chosen as the level of significance.

Results

Percentages of development from 1-cell embryos to each stage up to expanded blastocysts in 0.1 and 1.0 mg/ml PVA-supplemented media were comparable to those in the BSA-supplemented medium. However, percentages of development to partial hatching and complete hatching of blastocysts were significantly decreased in 0.1 mg/ml PVA-supplemented medium ($P<0.01$), and were moderately reduced in 1.0 mg/ml PVA-supplemented medium (Table 2). Percentages of development of 1-cell embryos to morulae and early blastocysts were significantly low in 10.0 mg/ml PVA-supplemented medium, compared with 0.1 and 1.0 mg/ml PVA-supplemented medium ($p<0.01$). No embryos in the 10.0 mg/ml PVA-supplemented medium developed to blastocysts. In 0.1 and 1.0 mg/ml PVA-supplemented medium, speeds of development from 1-cell embryos to each stage up to expanded blastocysts were the same as those in the BSA-supplemented medium (Table 3).

Table 2. Effect of concentration of polyvinyl alcohol (PVA) on the development of mouse 1-cell embryos

Conc. of PVA (mg/ml)	No. of embryos cultured	Percentage of embryos developed to						
		8[1)]	M[1)]	E-B[1)]	B[1)]	Exp-B[1)]	Part-HB[1)]	Comp-HB[1)]
0[2)]	52	100	100[a]	100[a]	100	100	44.2[a]	28.8[a]
0.1	64	100	100[a]	100[a]	100	100	20.3[b]	7.8[b]
1.0	65	100	100[a]	100[a]	100	100	27.7[ab]	15.4[ab]
10.0	54	100	68.5[b]	16.7[b]	0	0	0	0

[1)] 8: 8-cell, M: morula, E-B: early blastocyst, B: blasyocyst, Exp-B: expanded blastocyst, Part-HB: partially hatched blastocyst, Comp-HB: completely hatched blastocyst. [2)] Control: bovine serum albumin (BSA) 3 mg/ml. [a, b] Means with different superscripts within the same column are significantly different ($P<0.01$).

Table 3. Effect of concentration of polyvinyl alcohol (PVA) on the developmental speed of mouse 1-cell embryos

Conc. of PVA (mg/ml)	No. of embryos examined	Time required to develop to (h)[1)]								
		2[2)]	4[2)]	8[2)]	M[2)]	E-B[2)]	B[2)]	Exp-B[2)]	Part-HB[2)]	Comp-HB[2)]
0[3)]	15[4)]	16.2[a]	36.6	50.2	63.4	82.2	89.0	97.8	126.6	146.7
		± 4.6	± 3.4	± 4.3	± 4.9	± 6.0	± 6.1	± 6.1	± 19.5	± 22.3
0.1	5[4)]	15.0[a]	36.6	48.6	61.8	81.0	88.2	96.6	115.8	148.6
		± 6.2	± 2.6	± 2.6	± 0.4	± 2.8	± 3.5	± 2.6	± 7.6	± 37.5
1.0	10[4)]	17.3[a]	38.3	49.7	61.7	83.3	93.5	102.5	133.1	140.3
		± 5.3	± 2.1	± 2.9	± 0.5	± 7.6	± 5.7	± 5.9	± 12.1	± 13.4
10.0	9[5)]	24.3[b]	36.3	48.3	62.3	84.3				
		± 2.0	± 2.0	± 2.0	± 4.0	± 5.6				

[1)] Mean ± SD. [2)] 2: 2-cell, 4: 4-cell, 8: 8-cell, M: morula, E-B: early blastocyst, B: blasyocyst, Exp-B: expanded blastocyst, Part-HB: partially hatched blastocyst, Comp-HB: completely hatched blastocyst. [3)] Control: bovine serum albumin (BSA) 3 mg/ml. [4)] No. of embryos developed up to completely hatched blastocyst. [5)] No. of embryos developed up to early blastocyst. [a, b] Means with different superscripts within the same column are significantly different ($P<0.05$).

Table 4. Effect of polyvinyl alcohol (PVA) on development of mouse 2-cell, 8-cell embryos and early blastocysts

Stage of embryo cultured	Conc. of PVA (mg/ml)	No. of embryos cultured	Percentage of embryos developed to		
			Exp-B[1)]	Part-HB[1)]	Comp-HB[1)]
2[1)]	0[2)]	54	100	66.7[a]	44.4[a]
	1.0	58	100	20.7[b]	5.2[b]
8[1)]	0[2)]	56	100	82.1	60.7
	1.0	65	100	78.5	50.8
E-B[1)]	0[2)]	60	100	51.7	35.0[a]
	1.0	62	100	37.1	9.7[b]

[1)] Exp-B: expanded blastocyst, Part-HB: partially hatched blastocyst, Comp-HB: completely hatched blastocyst. [2)] Control: bovine serum albumin (BSA) 3 mg/ml. [a, b] Means with different superscripts within the same column are significantly different ($P<0.01$).

When 2-cell or 8-cell embryos or early blastocysts were cultured in 1.0 mg/ml PVA-supplemented medium, percentages of development to expanded blastocysts were comparable to the BSA-supplemented medium (Table 4). However, when 2-cell embryos were cultured in the PVA-supplemented medium, percentages of development from expanded blastocysts to partial hatching and complete hatching of blastocysts (20.7 and 5.2%, respectively) were significantly decreased, compared to percentages in the BSA-supplemented medium (66.7 and 44.4%, respectively; $p<0.01$). When 8-cell embryos and early blastocysts were cultured in the PVA-supplemented medium, these percentages tended to decrease (Table 4). In the PVA-supplemented medium, the developmental speeds of these embryos to each stage up to expanded

Table 5. Effect of polyvinyl alcohol (PVA) on developmental speeds of mouse 2-cell, 8-cell embryos and early blastocysts

Stage of embryo examined	PVA concentration (mg/ml)	No. of embryos examined[2)]	Time required to develop to (h)[1)]							
			4[3)]	8[3)]	M[3)]	E-B[3)]	B[3)]	Exp-B[3)]	Part-HB[3)]	Comp-HB[3)]
2[3)]	0[4)]	24	12.8 ± 0.4	25.5 ± 3.6	39.5 ± 5.2	60.0 ± 4.4	69.5 ± 5.1	78.0 ± 3.6	101.0 ± 11.4	112.8[a] ± 8.7
	1.0	3	12.7 ± 0.5	22.7 ± 5.2	38.7 ± 2.4	58.7 ± 5.2	64.7 ± 5.2	78.7 ± 0.5	102.7 ± 8.7	126.7[b] ± 18.0
8[3)]	0[4)]	34			12.7 ± 1.9	27.5[a] ± 5.1	34.6[a] ± 4.8	41.6[a] ± 4.4	70.6[a] ± 20.7	86.5 ± 25.0
	1.0	33			12.2 ± 1.0	22.7[b] ± 4.6	29.1[b] ± 4.5	36.5[b] ± 5.2	57.6[b] ± 10.5	80.9 ± 23.2
E-B[3)]	0[4)]	21					6.3 ± 1.3	17.1 ± 6.5	37.1 ± 12.6	59.1 ± 19.1
	1.0	6					7.0 ± 0.9	14.0 ± 1.2	46.0 ± 9.3	65.0 ± 8.2

[1)] Mean ± SD. [2)] No. of embryos developed up to completely hatched blastocyst. [3)] 2: 2-cell, 4: 4-cell, 8: 8-cell, M: morula, E-B: early blastocyst, B: blasyocyst, Exp-B: expanded blastocyst, Part-HB: partially hatched blastocyst, Comp-HB: completely hatched blastocyst. [4)] Control: bovine serum albumin (BSA) 3 mg/ml. [a, b] Means with different superscripts within the same column are significantly different ($P<0.01$).

blastocysts were the same as those in the BSA-supplemented medium, except when 8-cell embryos were cultured in the PVA-supplemented medium (Table 5).

Discussion

In the present study, 1-cell mouse embryos developed to expanded blastocysts in medium supplemented with 0.1 or 1.0 mg/ml PVA at a high percentage comparable to results for BSA-supplemented medium. This indicates that PVA at an optimum concentration supports embryo development from 1-cell to the expanded blastocyst stage, and that the PVA-supplemented medium can be a valuable chemically defined medium for mouse embryonic culture.

Biggers *et al*. [13] reported that incidence of development to the blastocyst stage was decreased when PVA was substituted for BSA in KSOM medium, and that percentage of development to the blastocyst stage was reduced to a greater extent in 1.0 mg/ml PVA-supplemented medium than in 0.1 mg/ml PVA-supplemented medium. In the present study, no embryos in 10.0 mg/ml PVA-supplemented medium developed to the blastocyst stage. Together, these results indicate that there is an optimum concentration of PVA supplementation of medium at which PVA supports embryo development.

Although a mouse embryo composed of a smaller number of blastomeres than a normal embryo can develop to the blastocyst stage [20], expansion of blastocysts depends on the number of cells [21]. However, there was no difference in total cell number between blastocysts developed in PVA-supplemented medium and those developed in BSA-supplemented medium [13]. In the present study, percentages and speeds of embryo development to each stage up to expanded blastocysts were almost the same for the PVA-supplemented medium as for the BSA-supplemented medium, even though cultures started at 1-cell, 2-cell, 8-cell or the early blastocyst stage. Together, these results suggest that embryos in the PVA-supplemented medium develop to expanded blastocysts via normal processes, and that PVA supports the embryo development in each stage up to the expanded blastocyst stage.

It has been reported that, for mouse [9, 13, 16], rabbit [14] and equine [15] embryos, the hatching rate of the blastocyst is decreased in PVA-supplemented media. In the present study, incidences of partial hatching and complete hatching of blastocysts were clearly decreased in the PVA-supplemented medium for culture of embryos from 1-cell to the early blastocyst stage. In recent studies using mouse embryos, trypsin-like protease activity in the medium gradually increased during the course of hatching [22], and trypsin-like protease localized in the mural trophectoderm [23, 24]. This suggests that protease participates in the hatching of blastocysts *in vitro* [22, 24]. Thus, the low hatching

rate of blastocysts in PVA-supplemented media may be due to a decline in protease synthesis and/or secretion.

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