应用乙二醇冷冻小鼠胚胎:优化和简化程序的探索

Cryopreservation of Mouse Embryos in Ethylene Glycol-based Solutions: A Search for the Optimal and Simple Protocols

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摘 要 提高解冻胚胎的发育能力和简化冷冻-解冻程序是胚胎冷冻研究的两大永恒的主题。尽管乙二醇(EG)广泛用于家畜胚胎冷冻,但很少用于冷冻小鼠和人胚胎。为数很少的以 EG 慢冻小鼠或人胚胎的研究均采用较为复杂的人胚冷冻程序,未见简化程序和用 EG 冷冻小鼠桑椹胚的报道。采用简单的牛胚胎冷冻程序研究了发育时期、EG 浓度、平衡方法、添加蔗糖以及解冻后脱除 EG 等对小鼠胚胎冻后发育能力的影响。结果显示:(1)致密晚期桑椹胚冻后体外培养囊胚发育率(81.92% ± 2.24%)和孵出率(68.56% ± 2.43%)显著($P < 0.05$)高于 4-细胞、8-细胞胚胎和致密早期桑椹胚胎;(2)1.8mol/L EG 冷冻小鼠致密晚期桑椹胚的囊胚发育和孵出率显著高于其它浓度;(3)在 EG 中平衡 10min 的冻后囊胚发育显著好于平衡 5、20 或 30min;(4)两步平衡冷冻胚胎的囊胚发育率和孵出率显著高于一步平衡;(5)用 EG 冷冻小鼠胚胎无需添加蔗糖;(6)解冻后可不脱除 EG;(7)冻后发育的早期囊胚和囊胚细胞数明显少于体内发育胚胎。因此,用 EG 冷冻小鼠胚胎的最佳方案为:致密晚期桑椹胚用 1.8mol/L EG 不添加蔗糖、两步平衡 15min、以简单的牛胚胎冷冻程序冷冻-解冻、解冻后不脱除 EG 直接培养或移植。

关键词 胚胎,冷冻,乙二醇,小鼠

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Abstract Although ethylene glycol (EG) has been widely used for embryo cryopreservation in domestic animals, few attempts were made to use this molecule to freeze mouse and human embryos. In the few studies that used EG for slow-freezing of mouse and human embryos, complicated protocols for human embryos were used, and the protocols need to be simplified. Besides, freezing mouse morula with EG as a cryoprotectant has not been reported. In this paper, we studied the effects of embryo stages, EG concentration, duration and procedure of equilibration, sucrose supplementation and EG removal after thawing on the development of thawed mouse embryos, using the simple freezing and thawing procedures for bovine embryos. The blastulation and hatching rates (81.92% ± 2.24% and 68.56% ± 2.43%, respectively) of the thawed late compact morulae were significantly ($P < 0.05$) higher than those of embryos frozen-thawed at other stages. When mouse late compact morulae were frozen with different concentrations of EG, the highest rates of blastocyst formation and hatching were obtained with 1.8mol/L

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EG. The blastulation rate was significantly higher when late morulae were equilibrated in 1.8mol/L EG for 10 min prior to freezing than when they were equilibrated for 30 min, and the hatching rate of embryos exposed to EG for 10 min was significantly higher than that of embryos exposed for 20 and 30 min. Both rates of blastocyst formation and hatching obtained with two-step equilibration were higher ($P < 0.05$) than with one-step equilibration in 1.8mol/L EG. Addition of sucrose to the EG-based solution had no beneficial effects. On the contrary, an increased sucrose level (0.4 mol/L) in the solution impaired the development of the frozen-thawed embryos. In contrast, addition of 0.1mol/L sucrose to the propylene glycol (PG)-based solution significantly improved the development of the frozen-thawed embryos. Elimination of the cryoprotectant after thawing did not improve the development of the thawed embryos. The cell numbers were less ($P < 0.05$) in blastocysts developed from the thawed morulae than in the *in vivo* derived ones. In summary, embryo stage, EG concentration, duration and procedure of equilibration and sucrose supplementation had marked effects on development of the thawed mouse embryos, and a protocol for cryopreservation of mouse embryos is recommended in which the late morulae are frozen in 1.8mol/L EG using the simple freezing and thawing procedures fo bovine embryos after a two-step equilibration and the embryos can be cultured or transferred without EG removal after thawing.

Key words embryo, cryopreservation, ethylene glycol, mouse

在用体外受精及相关技术治疗人类不孕症时,每次移植的胚胎数已由 4 枚减少到 2 枚[1]。而且,更趋于每次移植一枚囊胚[2]。因此,剩余胚胎的保存迫切需要高效胚胎冷冻技术。由于用人胚胎进行实验涉及伦理道德问题,故胚胎冷冻研究多用小鼠进行。过去,人和小鼠胚胎冷冻多采用丙二醇(1,2-propanediol, PG)[3-5]。目前,乙二醇(Ethylene glycol, EG)广泛应用于家兔[6]、牛[7]和绵羊[8]的胚胎冷冻。EG 对于动物胚胎的低毒性[7,9,10,11]和冷冻胚胎出生正常后代[11-13]都说明,它可以用于人的胚胎冷冻。尽管近年来已开始探索用 EG 冷冻小鼠胚胎,但这些研究均是采用较为复杂的人胚胎冷冻-解冻程序进行的,尚未见进行 EG 冷冻小鼠胚胎程序简化研究的报道。

以往对人和小鼠胚胎冷冻的研究多冷冻 2-~8-细胞胚胎,并取得了满意的效果[14-16]。然而,由于胚胎基因组一般在 4-~8-细胞以后才激活[17],故受精卵和早期卵裂胚胎发育到囊胚的能力很差,于是人们对囊胚冷冻也进行了大量研究[18-25]。然而,囊胚的冷冻效果并不好[19,20,22,23]。尽管就胚胎质量判定而言,桑椹胚不如囊胚,但还是比原核和早期卵裂胚胎好。

本实验采用比人胚冷冻程序更为简单的 EG 冷冻牛胚胎的冷冻程序研究了胚胎发育时期、EG 浓度、平衡方法及冷冻液中添加蔗糖对小鼠冷冻胚胎发育到囊胚和孵出囊胚的影响。同时,还比较了 EG 和 PG 对小鼠胚胎的冷冻保护能力以及解冻后脱出冷冻保护剂对冻胚发育的影响。为寻找更好的胚胎冷冻保护剂和进一步简化胚胎冷冻-解冻保存技术提供了必要的依据。

1 材料与方法

1.1 小鼠胚胎的获取

6~10 周龄昆明白雌鼠控光(14h 光照,10h 黑暗)饲养 1~2 周后,腹腔注射 10 IU/只 PMSG(宁波激素制品厂)和 hCG(宁波激素制品厂)进行超排。注射 hCG 后与雄鼠合笼,次日早晨检查阴道栓。

见栓雌鼠分别于注射 hCG 后 52~54h、65~66h、77~78h、80~82h、85~86h 和 90~91h 处死,采集 4-细胞、8-细胞、致密早期和晚期桑椹胚以及早期囊胚和囊胚。在实体显微镜下,8-细胞期胚胎细胞边缘清晰,未发生致密化(图 1C);致密早期桑椹胚的细胞开始相互紧靠,细胞边缘不十分清晰,但能区分每一个细胞(图 1E);致密晚期桑椹胚的细胞紧靠在一起,很难区分单个细胞(图 1G)[5]。

1.2 胚胎冷冻

冷冻液为含 3mg/mL BSA 的 M2 添加不同浓度的 EG(天津产)或 PG(天津产)。先将胚胎移入 0.9mol/L EG 中室温下平衡 5min,再移入 1.8mol/L EG 中平衡 10min,同时装入 0.25mL 塑料细管,每管 2 枚胚胎。将装有胚胎的塑料细管放入冷冻仪(日本富士平 FHK ET-1N)中,在 -7℃下平衡 2min 后植冰并平衡 8min。然后,以 -0.3℃/min 的速率降至 -30℃。在 -30℃下平衡 5min 后,将塑料细管投入液氮中保存。

1.3 胚胎解冻

胚胎冷冻保存 1 周后解冻。自液氮罐中取出塑料细管,室温下于空气中停留 10s,再置入盛有 32℃

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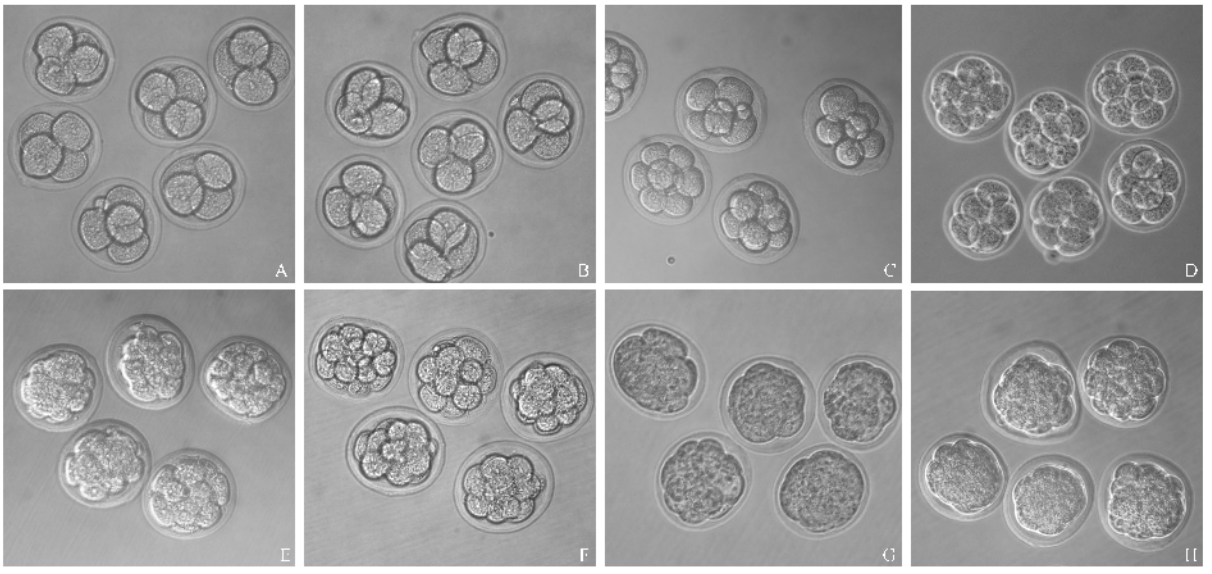

图 1 小鼠发育不同时期胚胎冷冻前后显微照片(×200)

Fig.1 Micrographs of mouse embryos before and after cryopreservation at different stages of development(×200)

A and B: 4-cell embryos before and after freezing; C and D: 8-cell embryos before and after freezing; E and F: early morulae before and after freezing; G and H: late morulae before and after freezing.

水浴的解冻杯中,停留 10s。取出细管,将解冻后的胚胎推入平皿中,再移入含 0.5mol/L 蔗糖的 M2 中 5min,最后用 M2 洗 3 次,以便脱除 EG。

1.4 胚胎培养

培养液为 CZB。于胚胎解冻前 2 h 在培养皿中制作 50μL 培养滴,覆盖石蜡油,放入 CO_2 培养箱中平衡。将解冻胚胎移入培养滴中,每滴 2 ~ 3 枚胚胎,放入 37.5℃、5% CO_2、饱和湿度的培养箱内培养并于不同时间观察记录囊胚发育率和孵出率。

1.5 胚胎细胞计数

将胚胎移入含 10μg/mL Hoechst33342 的 M2 中处理 5min,装片后在荧光显微镜下观察,记录胚胎细胞数。

1.6 统计分析

所有实验至少重复 3 次以上。实验数据利用 SPSS 统计软件(SPSS Inc.)的 ANOVA 模块分析。数据先经 LSD 转换后,再进行单因素方差分析,$P < 0.05$为差异显著。

2 结果

2.1 不同发育阶段小鼠胚胎 EG 冷冻后的发育

将 4-细胞、8-细胞、致密早期和晚期桑椹胚在 0.9mol/L EG 中平衡 5min,再移入 1.8mol/L EG 中平衡 10min,装入 0.25mL 塑料细管中冷冻。4-细胞和 8-细胞胚胎放入冷冻液后卵裂球因脱水而收缩,细胞间距离和卵周隙增大(图 1B 和 D)。致密早期桑椹胚放入冷冻液后,细胞间界限更清晰(图 1F)。致密晚期桑椹胚在冷冻液中体积变小,但细胞不分离(图 1H)。解冻脱除冷冻液后,大部分胚胎能恢复到冷冻前的状态,但细胞质内常出现小颗粒。

冻后胚胎体外培养结果(表 1)显示,致密晚期桑椹胚的囊胚发育率(81.92% ± 2.24%)和孵出率(68.56% ± 2.43%)均显著($P < 0.05$)高于其他时期胚胎。4-细胞、8-细胞和致密早期桑椹胚的囊胚率差异不显著,但 8-细胞胚胎的囊胚孵出率显著高于 4-细胞和致密早期桑椹胚。

2.2 EG 浓度对冷冻-解冻胚胎发育的影响

致密晚期桑椹胚在 0.9mol/L EG 中平衡 5min 后,移入 0.9、1.8、2.7 和 3.6mol/L EG 中平衡 10 min 后冷冻。解冻后体外培养的囊胚发育率和囊胚孵出率以 1.8mol/L 的最高(80.83% ± 2.20% 和 75.00% ± 2.04%),极显著($P < 0.01$)高于其它组(表 2)。

2.3 平衡时间对冷冻小鼠胚胎发育的影响

致密晚期桑椹胚在 0.9mol/L EG 中平衡 5min,移入 1.8mol/L EG 中平衡不同时间后冷冻。在 1.8mol/L EG 中平衡 10min 的囊胚发育率(80.97% ± 4.14%)显著($P < 0.05$)高于平衡 30min 的(66.35% ± 1.45%),囊胚孵出率(76.77% ± 1.84%)显著高于平衡 20 和 30min(70.15% ± 1.37% 和 60.75% ± 3.77%)(表 3)。说明平衡时间可以在 5 ~ 10min,但不宜过长。

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表 1 不同发育阶段小鼠胚胎 EG 冷冻后的发育

Table 1 Development of mouse embryos frozen with EG at different developmental stages

Stages of development	Embryos frozen	Embryos developing to/%	
		Blastocysts	Hatched blastocysts
4-cell	128	86(65.86 ± 1.44)[a]	76(57.56 ± 2.94)[ab]
8-cell	143	101(70.05 ± 1.43)[a]	88(61.07 ± 1.82)[b]
Early compact morulae	149	102(68.30 ± 0.71)[a]	82(54.36 ± 2.05)[a]
Late compact morulae	119	97(81.92 ± 2.24)[b]	84(68.56 ± 2.43)[c]

a,b,c:Values in the same column with a common letter in superscripts did not differ($P > 0.05$). The same for the following tables.

表 2 不同浓度 EG 冷冻小鼠致密晚期桑椹胚的发育

Table 2 Development of mouse late compact morulae after cryopreservation in different concentrations of EG

EG Concentrations/(mol/L)	Embryos frozen	Embryos developing to/%	
		Blastocysts	Hatched blastocysts
0.9	73	43(56.97 ± 2.52)[a]	37(50.14 ± 1.09)[a]
1.8	72	58(80.83 ± 2.20)[b]	54(75.00 ± 2.04)[b]
2.7	45	29(64.17 ± 2.50)[a]	19(42.50 ± 2.50)[c]
3.6	63	34(55.14 ± 3.54)[a]	13(20.97 ± 2.43)[d]

表 3 平衡不同时间 1.8mol/L EG 冷冻小鼠致密晚期桑椹胚的发育

Table 3 Development of mouse late compact morulae equilibrated in 1.8 mol/L EG for different times and frozen in liquid nitrogen

Time of equilibration/min	Embryos frozen	Embryos developing to/%	
		Blastocysts	Hatched blastocysts
5	62	48(78.30 ± 2.12)[a]	46(73.72 ± 1.34)[ab]
10	59	47(80.97 ± 4.14)[ab]	45(76.77 ± 1.84)[a]
20	67	48(71.81 ± 0.88)[bc]	47(70.15 ± 1.37)[bc]
30	69	46(66.35 ± 1.45)[d]	42(60.75 ± 3.77)[cd]

2.4 两步和一步平衡冷冻小鼠胚胎的发育

致密晚期桑椹胚在 0.9mol/L EG 中平衡 5min,移入 1.8mol/L EG 中平衡 10min,或者直接在 1.8 mol/L EG 中平衡 10min 后冷冻。结果表明,两步平衡冷冻胚胎的囊胚发育率(80.83% ± 2.20%)显著($P < 0.05$)高于一步平衡(72.64% ± 1.05%);前者的囊胚孵出率(75.00% ± 2.04%)极显著($P < 0.01$)高于后者(60.14% ± 2.36%)。

2.5 EG 冷冻液中添加不同浓度蔗糖对解冻胚胎发育的影响

致密晚期桑椹胚在 0.9mol/L EG 中平衡 5min,移入添加 0、0.05、0.1、0.2 和 0.4 mol/L 蔗糖的 1.8mol/L EG 中平衡 10min 后冷冻。添加 0、0.05 和 0.1 mol/L 蔗糖冷冻胚胎的囊胚发育率(80.83% ± 2.20%、78.00% ± 2.00% 和 80.41% ± 1.92%)和囊胚孵出率(75.00% ± 2.04%、70.67% ± 3.23% 和 74.32% ± 2.06%)差异不显著($P > 0.05$)。但添加 0.2 和 0.4mol/L 蔗糖的囊胚发育率(73.33% ± 2.11 和 57.08% ± 2.19%)和孵出率(62.67% ± 4.00% 和 43.61% ± 2.75%)显著($P < 0.05$)下降(表 4)。

表 4 在 1.8mol/L EG 中添加不同浓度蔗糖冷冻小鼠致密晚期桑椹胚的发育

Table 4 Development of mouse late compact morulae after cryopreservation in 1.8 mol/L EG supplemented with different concentratins of sucrose

Sucrose concentration/(mol/L)	Embryos frozen	Embryos developing to/%	
		Blastocysts	Hatched blastocysts
0	72	58(80.83 ± 2.20)[a]	54(75.00 ± 2.04)[a]
0.05	55	43(78.00 ± 2.00)[ab]	39(70.67 ± 3.23)[ab]
0.1	58	47(80.41 ± 1.92)[a]	43(74.32 ± 2.06)[a]
0.2	75	55(73.33 ± 2.11)[b]	47(62.67 ± 4.00)[b]
0.4	56	32(57.08 ± 2.19)[c]	24(43.61 ± 2.75)[c]

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2.6 解冻后脱除 EG 培养对胚胎发育的影响

致密晚期桑椹胚在 0.9mol/L EG 中平衡 5min,移入 1.8mol/L EG 中平衡 10min 后冷冻。胚胎解冻后移入含 0.5mol/L 蔗糖的 M2 中停留 5min,再用 M2 洗 3 次,脱除 EG。结果表明,脱除 EG 与不脱除 EG 的囊胚发育率(80.83% ± 2.20% 比 81.67% ± 3.19%)和囊胚孵出率(75.00% ± 2.04% 比 73.33% ± 2.72%)差异均不显著($P > 0.05$)。

2.7 EG 与 PG 冷冻小鼠胚胎的发育效果比较

致密晚期桑椹胚在 0.9mol/L EG 或 0.75mol/L PG 中平衡 5min,移入含有或不含有 0.1mol/L 蔗糖的 1.8mol/L EG 或 1.5mol/L PG 中平衡 10min 后冷冻。不添加蔗糖时,EG 和 PG 冷冻胚胎的囊胚发育率(80.83% ± 2.20% 对 86.39% ± 2.37%)和孵出率(75.00% ± 2.04% 对 79.84% ± 0.73%)差异不显著($P > 0.05$),但添加蔗糖时,PG 冷冻胚胎的囊胚发育率(87.64% ± 1.12%)和孵出率(83.47% ± 2.16%)显著高于 EG 冷冻胚胎(80.41% ± 1.92% 和 74.32% ± 2.06%)。用 PG 添加蔗糖冷冻致密晚期桑椹胚效果好于 EG(表 5)。

2.8 EG 冷冻胚胎体外发育囊胚的细胞数

以最佳方案(1.8mol/L EG 不添加蔗糖、两步平衡、解冻后脱除 EG)冷冻小鼠致密晚期桑椹胚发育产生的早期囊胚(29.00 ± 0.68)和囊胚(33.90 ± 0.85)的细胞数明显少于体内胚胎(30.93 ± 0.51、38.70 ± 0.76)。

表 5 EG 与 PG 冷冻小鼠致密晚期桑椹胚的发育

Table 5 Development of mouse late compact morulae after cryopreservation with EG or PG

Cryoprotectants	Embryos frozen	Embryos developing to/%	
		Blastocysts	Hatched blastocysts
EG-Sucrose	72	58(80.83 ± 2.20)[a]	54(75.00 ± 2.04)[a]
EG + Sucrose	58	47(80.41 ± 1.92)[a]	43(74.32 ± 2.06)[a]
PG-Sucrose	70	60(86.39 ± 2.37)[ab]	56(79.84 ± 0.73)[ab]
PG + Sucrose	64	56(87.64 ± 1.12)[b]	53(83.47 ± 2.16)[b]

3 讨论

过去,小鼠和人的冷冻胚胎常用 PG 作为保护剂,但最近证明,EG 毒性较小[7,9,11,15],并且已广泛用于动物胚胎的慢速[7,26]和玻璃化冷冻[9,10,13]。Van den Abbeel 等[27]和 Sommerfeld 和 Niemann[9]报道,慢速冷冻胚胎的效果要好于快速或超快速冷冻。然而,目前用 PG 或 EG 慢速冷冻小鼠胚胎的研究均采用较为复杂的人胚胎冷冻-解冻程序进行[5,15,27],尚未见到简化该程序的研究报道。本实验采用牛胚胎的冷冻程序[26],冷冻和解冻程序都大大简化。而且,我们还证明,解冻后可以不脱除 EG 进行胚胎培养。我们获得的小鼠致密晚期桑椹胚和 4-细胞胚胎的冻后囊胚发育率与 Tao 等[5]和 Emiliani 等[15]用人胚冷冻—解冻程序所获得的十分接近。说明可以用简化的冷冻-解冻程序进行小鼠胚胎 EG 冷冻。有人采用一步平衡冷冻牛胚胎成功[26],但本实验未能获得满意结果。另外,进行小鼠胚胎冷冻多在一个细管内装 15 ~ 20 枚胚胎[5,15]。这对于进行研究是方便的,但不实用,因为人和大动物每次一般移植 1 ~ 2 枚胚胎。本实验中每只细管装 2 枚胚胎冷冻也取得了满意的效果。

Shaw 等[14]发现,EG 慢冻 4-细胞胚胎效果好于 PG。Emiliani 等[15]发现,EG 慢冻 4-细胞胚胎效果最好,PG 冷冻原核胚效果最好,甘油冷冻囊胚效果最好。Bafrani 等[16]也发现,EG 不如 PG 更适合于小鼠合子冷冻。然而,这些实验并没有涉及 8-细胞和桑椹胚胎。本实验首次发现,用 EG 冷冻致密晚期桑椹胚的囊胚发育率和孵出率均显著高于其它时期胚胎;8-细胞胚胎的囊胚孵出率显著高于 4-细胞和致密早期桑椹胚。综上所述,用 EG 慢冻小鼠胚胎的效果具有明显的胚胎发育阶段依赖性:合子 < 4-细胞、致密早期桑椹和囊胚 < 8-细胞胚 < 致密晚期桑椹胚。Tao 等[5]用 PG 冷冻小鼠胚胎发现,冷冻致密晚期桑椹胚的囊胚发育率显著高于 2-细胞胚,而后者的又显著高于合子和致密早期桑椹胚。这表明,(1)对于不同发育阶段胚胎,必须采用不同的冷冻程序和保护剂才能收到满意的冷冻效果;(2)致密晚期桑椹胚最耐冻,而致密早期桑椹胚对现有冷冻程序,特别是 PG 冷冻更敏感。关于致密早期桑椹胚对冷冻敏感的原因可能有三。第一,这些胚胎发育速度慢,为劣质胚胎。第二,由于致密早期桑椹胚细胞间建立了部分连接,故在进入冷冻液后因细胞皱缩而使膜受到牵拉而易受损。第三,不同发育时期胚胎的细胞膜对不同保护剂的通透性不同,在 1.8mol/L EG 中致密早期桑椹胚卵裂球相互分离,致

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密晚期桑椹胚卵裂球仍然紧密排列。由于本实验中严格控制采胚时间,致密早期和晚期桑椹胚并非同时获得,故可排除第一种可能性。本实验中,致密早期桑椹胚的冷冻效果与4-和8-细胞胚胎相似也可排除第二种可能性。而本实验和众多研究所证明的用EG慢冻小鼠胚胎的效果具有明显的胚胎发育阶段依赖性却明显支持第三种可能性。

本实验应用0.9、1.8、2.7和3.6 mol/L EG冷冻小鼠致密晚期桑椹胚,解冻后体外培养的囊胚发育率和孵出率以1.8 mol/L的最高,极显著($P < 0.01$)高于其它组。本实验中,胚胎在1.8mol/L EG中平衡5~10min的囊胚发育率显著高于平衡30min,平衡10min的囊胚孵出率显著高于平衡20~30min。说明在EG中的平衡具有严格的时限,10min最好。他人对其他冷冻保护剂的浓度[28]和平衡时间[24,29]的研究也得到了相似的结论。

尽管目前在用EG冷冻牛胚胎时一般不添加蔗糖,但Martinez等[30]研究证明,EG添加0.1mol/L蔗糖冷冻牛胚胎的妊娠率显著高于不添加或添加0.3mol/L蔗糖。我们发现,在含1.8 mol/L EG的冷冻液中添加0、0.05和0.1 mol/L蔗糖冷冻小鼠胚胎的囊胚发育率和孵出率差异不显著,但添加0.2或0.4mol/L蔗糖的囊胚发育率和孵出率显著下降。Dochi等[26]报道,用1.6mol/L PG冷冻牛囊胚和桑椹胚的效果不如用1.8mol/L EG。本实验比较PG和EG对小鼠致密晚期桑椹胚的冷冻效果发现,在不添加蔗糖的情况下,EG和PG冷冻的囊胚发育率和孵出率差异不显著,但在添加0.1 mol/L蔗糖时,PG冷冻胚胎的囊胚发育率和孵出率显著高于EG冷冻胚胎。这说明,用EG冷冻小鼠桑椹胚时无需添加蔗糖,但用PG冷冻时最好添加蔗糖。PG和EG作为冷冻保护剂对蔗糖的需求差别可能与所用的胚胎解冻方法有关。Shaw等[14]发现,只有用PG添加0.1mol/L蔗糖冷冻小鼠原核和4-细胞胚用多种解冻方法都能取得好效果,而用PG不加蔗糖、EG加或不加蔗糖冷冻时,只有用一种(与本实验完全相同的)解冻方案,才获得好效果。

本实验发现,冻后发育的早期囊胚和囊胚的细胞数明显少于体内发育的早期囊胚和囊胚。这说明,经现有方法冷冻-解冻胚胎仍会损伤部分细胞,影响胚胎发育能力。形态观察也发现,解冻后胚胎虽然能恢复到冷冻前的大小,但细胞质出现微小的颗粒。说明经过冷冻-解冻后,细胞已受到损伤。

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