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人早期胚胎解冻后氨基酸代谢变化的临床分析*

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摘要 目的:分析人早期胚胎解冻后的氨基酸代谢变化,以明确冻融胚胎解冻后的最佳移植时机。**方法:**收集 2013 年 1 月-2014 年 1 月本中心 24 例经体外受精-胚胎移植治疗患者取卵后第 3 日 6~8 细胞废弃胚胎,采用囊胚培养液微滴对其培养,培养 2h 后用玻璃化冷冻保存,于冷冻前 2h、解冻后各时段分别收集 15 μ L 胚胎培养液微滴,以同一培养皿中未进行胚胎培养的培养液为对照组。通过高效液相色谱法检测标本在不同时段(0.5、1、2、4、6、24 h)的氨基酸浓度变化。**结果:**对照组解冻后不同时间点赖氨酸、色氨酸、组氨酸与谷氨酰胺浓度与解冻前比较,差异均具有统计学意义($P<0.05$),其余 16 种氨基酸解冻前后浓度无明显变化,差异均无统计学意义($P>0.05$)。胚胎培养液解冻后 1 h,胚胎培养液中的氨基酸浓度均明显高于对照组,差异具有统计学意义($P<0.05$),氨基酸转量明显高于解冻前、解冻后 0.5 h、4 h、6 h,差异具有统计学意义($P<0.05$)。**结论:**人早期胚胎在解冻后约 0.5h 就已开始恢复代谢状态,已开始进行氨基酸代谢,且氨基酸代谢水平与冷冻前基本相同。

关键词:冻融胚胎移植;移植时机;氨基酸代谢;高效液相色谱法

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Analysis on Changes in Amino Acid Metabolism after Human Early Embryos*

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ABSTRACT Objective: To detection and analysis on changes in amino acid metabolism after human early embryos, in order to define the best opportunity for the transplantation of frozen thawed embryos. **Methods:** Selected 24 cases of third days after fertilization and embryo transfer treatment in patients with 6~8 cell abandoned embryos who treated in the center from January 2013 to January 2014, the blastocyst culture solution microdroplet was applied to culture such embryos, vitrification cryopreservation was taken after 2 hours of culture, 15 μ L of embryo culture droplets were respectively collected at time frames of 2 h before freezing and after thawing, embryos in the culture solution in the same petri dish bypassing embryo culture were selected into the control group. The changes in amino acid concentration were detected at different times via performance (0.5, 1, 2, 4, 6, 24 h) by high performance liquid chromatography. **Results:** In the control group, the concentration of lysine, tryptophan, histidine and glutamine after thawing at the different time points were statistically significant compared with before thawing ($P<0.05$), the remaining 16 amino acids before and after thawing concentration had no significant changes, the difference were not statistically significant ($P>0.05$), 1 h after thawing, the concentrations of amino acids in embryo culture medium were significantly higher than those in the control group, the differences were statistically significant ($P<0.05$), thawing amino conversion were significantly higher than that of before thawing, 0.5 h, 4 h, and 6 h, the differences were statistically significant ($P<0.05$). **Conclusion:** Human early embryos have begun to recover about 0.5 h after thawing, amino acid metabolism has been started, and its metabolism of amino acid is basic same to that before thawing.

Key words: Freezing and thawing embryo transfer; Transplant time; Amino acid metabolism; High performance liquid chromatography

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前言

冻融胚胎移植通过冷冻保存控制性促排卵周期中优质的胚胎,并对其进行解冻移植,以增加控制性促排卵周期的妊娠

率,是作为体外受精-胚胎移植技术的补充^[1,2]。虽然冻融胚胎移植技术增加了患者的妊娠率,减轻了患者的心理负担,但与新鲜周期胚胎移植比较,其成功率较低^[3]。冻融胚胎质量、胚胎移植的数目、冻融胚胎复苏时间等均是影响冻融胚胎移植成功

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的主要原因^[9]。基于此,我们通过对人早期胚胎解冻后氨基酸代谢水平进行分析,以探讨人早期胚胎解冻后氨基酸代谢规律,为胚胎解冻后的移植提供可靠依据。

1 资料与方法

1.1 一般资料

选取 2013 年 1 月 -2014 年 1 月于本院生殖中心 24 例体外受精 - 胚胎移植患者取卵后第 3 日 6~8 个细胞的废弃胚胎作为本次研究对象。患者年龄 23~38 岁,平均(29.3± 3.1)岁;不孕时间 3~7 年,平均(4.7± 1.2)年;胚胎冷冻时间 5~11 d,平均(7.4± 0.8)d;不孕原因:子宫内膜异位症 9 例,盆腔输卵管异常 15 例。所有患者均对本次研究知情且签署知情同意书。

1.2 研究方法

首先,将挑选好的胚胎移至 CO₂ 平衡好的 20 μL 囊胚培养液(美国 Sage 公司)微滴中单独培养 2 h 后行玻璃化冷冻保存,收集培养过胚胎的培养液微滴 15 μL 作为观察组,同时收集同一培养皿中未培养过胚胎的培养液作为空白对照组,并及时将以上处理好的标本置于 -80℃ 的冰箱中保存待检测。随后,将冰箱中的观察组及空白对照组的标本取出,将其放于干冰中,运送到分析测试中心进行高效液相色谱检测。冷冻胚胎置于常温

环境下进行解冻,采用由 Agilent 公司生产的 1100 型高效液相色谱仪对解冻胚胎进行检测,检测方法为柱前衍生反相 HPLC 检测。氨基酸浓度由氨基酸标准品与色谱图曲线面积对照所得。不同时间点(解冻后 0.5、1、2、4、6、24 h)胚胎培养液中氨基酸的浓度减去对照组氨基酸浓度即可得到不同时间点培养液中氨基酸的浓度。所得数值为负数则表示培养液中氨基酸浓度较对照组降低;所得数值为 "0" 则表示氨基酸浓度无变化;所得数值为正数则表示氨基酸浓度增高^[9]。氨基酸浓度增加量加上氨基酸减少量的绝对值为氨基酸转换量^[9]。

1.3 统计学处理

采用 SPSS17.0 软件包进行统计学分析。计量数据描述采用均数± 标准差,比较采用 t 检验和方差齐性分析,P<0.05 表示差异有统计学意义。

2 结果

2.1 对照组解冻前后不同时间点的氨基酸浓度变化

对照组解冻前赖氨酸、色氨酸、组氨酸与谷氨酰胺浓度与解冻后不同时间点(0.5、1、2、4、6、24 h)比较,组间差异均具有统计学意义(P<0.05),其余 16 种氨基酸解冻前后浓度无明显变化,差异均无统计学意义(P>0.05)。见表 1。

表 1 对照组解冻前与解冻后不同时间点的氨基酸浓度变化(μmol/L)

Table 1 Comparison of the amino acid concentration of before thawing and different time points after thawing in the control group (μmol/L)

Types	Before thawing	0.5 h after thawing	1 h after thawing	2 h after thawing	4 h after thawing	6 h after thawing	24 h after thawing
Glutamate	2.4*	1	1	1.4	1	1	1
Serine	94	98	94	94	100	102	106
Glycine	88.4	98	92	92.4	98	99	100
Histidine	158.4*	197	173	174	181	186	192
Asparagic acid	98	103	103	105.4	102	106	117
Asparagine	88.4	106	95	90	91	98	101
Alanine	15.6	11	15	15	15	16	17
Taurine	84	106	101	97	101	103	105
Arginine	598	621	578	575	582	576	598
Valine	169	203	177	170.6	174	181	192
Tryptophan	74*	101	85	83	92	91	93
Methionine	74	101	85	83	92	91	95
Leucine	374.1	418	372	375	386	394	401
Phenylalanine	178.6	205	178	179.6	188	192	194
Lysine	325.1*	413	371	358	377	395	403
Isoleucine	362.4	402	355	357	370	382	387
Tyrosine	167	202	176	171.5	177	181	190
Alanyl-glu-tamine	835	953	861	840	911	898	937
Threonine	379.5	406	364	368.4	375	187	397

Note: Compared with different time points after thawing, *P<0.05.

2.2 胚胎培养液解冻前后不同时间点的氨基酸浓度变化及氨基酸转换情况

解冻后 1h 时,胚胎培养液中的氨基酸浓度均明显高于对

照(0 μmol/L),差异具有统计学意义(P<0.05);解冻后 1 h 氨基酸转换量明显高于解冻前、解冻后 0.5 h、4 h、6 h,差异具有统计学意义(P<0.05)。见表 2。

表 2 胚胎培养液解冻前后不同时间点的氨基酸浓度变化及氨基酸转换情况($\mu\text{mol/L}$)Table 2 Change of amino acid concentrations before thawing and in different time points after thawing of embryo culture solution and conversion situation of amino acid($\mu\text{mol/L}$)

Types	Before thawing	0.5 hour after thawing	1 h after thawing	2 h after thawing	4 h after thawing	6 h after thawing	24 h after thawing
Glutamate	2	0	0*	0	-2	-1	2
Serine	4	-1	15*	3	1	3	-15
Glycine	5	-3	8*	8	1	2	-1
Histidine	13	-2	12*	15	1	-2	-5
Asparagic acid	8	-3	8*	6	2	0	-1
Asparagine	-1	-1	13*	1	-2	-2	-14
Alanine	3	0	3*	3	3	4	35
Taurine	5	-2	5*	5	3	1	-2
Arginine	-2	-12	17*	26	-7	-21	-27
Valine	6	-8	23*	22	-2	-6	-16
Tryptophan	0	-2	3*	0	-1	-2	-3
Methionine	5	1	6*	5	-2	-2	-3
Leucine	7	-8	21*	18	-2	-5	-17
Phenylalanine	5	-4	11*	12	2	1	-6
Lysine	26	-5	21*	20	1	-2	-14
Isoleucine	6	-8	20*	21	-3	13	-17
Tyrosine	2	-16	8*	11	-2	-3	-8
Alanyl-glu-tamine	45	-51	47*	52	-4	-41	-70
Threonine	2	2	3*	3	5	3	30
Amount of amino acid conversion	147 [#]	129 [#]	244	231	46 [#]	114 [#]	286

Note: Compared with control group, * $P<0.05$, Compared with 1h after thawing, [#] $P<0.05$.

3 讨论

冻融胚胎移植是辅助生殖技术中的重要组成部分,明确冻融胚胎复苏与胚胎解冻后体外培养时间以确定最为适宜的移植时机可提高冻融胚胎移植的成功率^[7,8]。目前,生殖中心主要是以取卵后第3 d的解冻胚胎在体外培养3 h左右后实施移植。国外有学者^[9-11]经研究发现,人早期胚胎在解冻后于体外培养20 h后再进行移植可提高胚胎种植成功率,并提出解冻后增加培养时间,可挑选出质量相对较好的胚胎。但其是以取卵后第5 d的囊胚作为研究对象,囊腔会随着时间的推移而扩张,增加了胚胎孵出的机率,从而提高了囊胚种植的成功率。同时较多研究^[12-14]表明,适当延长取卵后第3 d冻融胚胎的培养时间,尽量选择分裂生长已基本恢复的胚胎进行移植可有效提高妊娠率。不过上述研究中所选取的胚胎在解冻后存活的胚胎数明显多于用于移植的胚胎数,研究只能说明选择具有继续发育能力的胚胎进行移植可提高冻融胚胎移植成功率,胚胎质量影响冻融胚胎移植成功率。目前,体外培养环境还未完全达到如子宫内的环境,胚胎体外培养环境的改善对冻融胚胎移植的影响值得国内外研究者给予关注^[15]。

在胚胎的发育过程中,各种氨基酸起着重要的作用,其代谢影响着胚胎的发育。通过HPLC法检测培养液中的氨基酸浓度,以获得氨基酸转换量。氨基酸转换量是评估临床妊娠率与活产率的重要指标^[16,17]。在本次研究中,笔者采用HPLC法检测人早期胚胎解冻前与解冻后各时间点培养液中的氨基酸水平,并以未进行胚胎培养的培养液作为空白对照,避免了由培养环境所带来的影响,结果表明在解冻前与解冻后不同时间点对照组赖氨酸、组氨酸、谷氨酰胺与色氨酸浓度比较,差异显著,具有统计学意义($P<0.05$),但这四种氨基酸浓度并未随着培养时间的延长而增加;其余氨基酸在解冻前与解冻后不同时间点浓度比较,差异无统计学意义($P>0.05$),由此说明,胚胎培养放置在培养液中的时间 <24 h并不会发生成分改变,这提示我们培养液中氨基酸浓度变化与胚胎代谢有关。氨基酸转换量体现了胚胎氨基酸的代谢能力^[18-20]。本研究发现,胚胎在解冻1 h后,胚胎培养液中氨基酸转化量明显高于解冻后0.5 h、4 h、6 h的转换量,这是由于人早期胚胎在解冻后置于体外胚胎培养液中培养时,为了适应环境的改变会代谢出较多的氨基酸,所以氨基酸浓度会呈一过性上升。胚胎培养液在胚胎解冻半小时后,其中氨基酸转换量与解冻前比较,差异无统计学意义,说

明胚胎在解冻半小时后即恢复了氨基酸代谢能力。

综上所述,人早期胚胎在解冻后约 0.5 h 就已开始恢复代谢状态,已开始进行氨基酸代谢,且氨基酸代谢水平与冷冻前基本相同;人早期胚胎解冻后并不一定需要在胚胎培养液中培养 2~4 h 后再进行移植,可提前移植,有助于培养时间的缩短,减少培养环境对胚胎带来的影响,但提前移植能否提高妊娠率还有待研究。

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