透明带激光削薄法在玻璃化

移植胚胎移植中的作用

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【摘要】目的：为探讨激光削薄法对冻融胚胎移植结局的影响。方法：选取分裂期胚胎移植组372个周期，囊胚期胚胎移植组81个周期以及反复种植失败(既往移植失败≥2次)移植组128个周期，分别按解冻单、双日将周期解冻胚胎分为激光削薄组和对照组，激光削薄组胚胎于移植前行卵透明带激光削薄处理，对照组胚胎不进行削薄处理，分析比较各组间的实验室和临床效果。

结果：移植分裂期胚胎组患者的生化妊娠率、临床妊娠率及胚胎移植率激光削薄组与对照组相比无统计学差异(49.11% vs 48.28%, 42.01% vs 42.36%, 28.66% vs 28.35%, P>0.05)；囊胚期胚胎激光削薄组患者的上述移植结局与对照组比较亦无统计学差异(60.47% vs 63.16%, 48.84% vs 55.26%, 37.88% vs 38.57%, P>0.05)；反复种植失败患者的冻融胚胎经激光削薄法处理后没有改善其妊娠结局(43.33% vs 45.59%, 36.67% vs 39.71%, 25.23% vs 26.15%, P>0.05)，但是在囊胚期胚胎和反复种植失败患者中，患者移植后的流产率激光削薄组较对照组有增高的趋势(19.05% vs 4.76%, P=0.15; 36.36% vs 11.11%, P=0.035)，其中反复种植失败激光削薄组显著高于对照组(P<0.05)。结论：透明带激光削薄辅助孵化技术并不能有效改善冻融胚胎移植患者的妊娠结局，其远期安全性有待于进一步研究。

关键词：激光；辅助孵化(AH)；玻璃化冷冻；胚胎移植(ET)

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透明带较厚患者的临床妊娠率和种植率。但是，也有许多研究者对此持反对意见，认为移植前对胚胎进行AH操作对患者的妊娠结局无明显改善。首先，可能会增加单卵双胎和异位妊娠的发生率。介乎AH技术的应用尚存在众多争议，为了进一步确认激光AH法在辅助生殖技术中的应用效果，本研究将探讨透明质酸激光切片法对玻璃化FET患者妊娠结局的影响。为激光AH技术的安全性和有效性评估提供理论依据。

1 材料与方法

1.1 研究对象

选取2011.07~12期间在本院实施FET的患者。按冷冻胚胎妊娠数进行分组，妊娠数为单日的周期数为对照组，妊娠数的胚胎不进行激光切片处理；妊娠数为单日的周期数为激光切片组。所妊娠的胚胎均进行激光切片处理。本研究方案经伦理委员会批准，所有患者均签署知情同意书。

1.2 胚胎玻璃化冷冻

胚胎的玻璃化冷冻操作均在室温下进行，先将胚胎移入冷冻平衡液(equilibration solution, ES, 美国Origio)(含体积分数20%血清替代物(serum substitute supplement, SSS)；体积分数7.5%乙二醇(ethylene glycol, EG)；体积分数7.5%二甲基亚砜(dimethyl sulphoxide, DMSO)的人输卵管液(modified human tubal fluid, mHTF))中充分平衡后转入玻璃化冷冻液(vitrification solution, VS, 美国Origio)(含体积分数20% SSS、体积分数15%EG、体积分数15% DMSO, 0.58 mol/L 蔗糖的mHTF)中，45~60 s后迅速将胚胎恢复至自制的冷冻片上，直接投入液氮中保存。胚胎在ES中的平衡时间依据胚胎体积恢复程度，一般以体积恢复至冷冻前80%为平衡充分。

1.3 胚胎复苏

胚胎解冻操作均在室温下进行，解冻前预先将解冻2液(含体积分数20% SSS, 0.83 mol/L 蔗糖的mHTF, 美国Origio)在37 °C 培养箱中预热。解冻2、3、4液于室温下平衡。解冻时，将装有胚胎的Strawtop自液氮中取出并迅速将其移入解冻1液中平衡1 min，随后将其转入解冻2液(含体积分数20% SSS, 0.42 mol/L 蔗糖的mHTF, 美国Origio)中平衡3 min，再依次在解冻3液(含体积分数20% SSS, 0.30 mol/L 蔗糖的mHTF, 美国Origio)、解冻4液(含体积分数20% SSS的mHTF, 美国Origio)液中平衡各5 min，最后将胚胎移入含体积分数10% SSS的胚胎培养液(multiblast medium, 美国Irvine Scientific)中，置于37 °C、体积分数5%CO2及饱和湿度的培养箱中培养。

1.4 激光切片法AH

将解冻胚胎置于倒置显微镜下，选择胚胎碎片较多或卵周隙狭小的透明带区域进行操作，轻轻移动载物台，待胚胎透明带区域与激光发射圈重叠，选择合适的激光强度依次发射激光进行切片。孵化区域约为1/4透明带周长、深度约为透明带厚度的70%~80%。孵化操作结束后，将胚胎移入37 °C，体积分数5%CO2及饱和湿度的培养箱中培养，等待移植。

1.5 胚胎移植与妊娠结果判断

胚胎移植的内膜准备主要有自然周期、激素替代及促排卵周期3种方式。按本单位常规进行。胚胎移植操作均在超声监测下进行，移植后14 d进行尿hCG检测。阳性患者定为生化妊娠。再经14 d后进行阴道超声检查，见孕囊者定为临床妊娠。

1.6 统计学处理

结果以均数±标准差(x±s)或率(%)表示。应用SPSS20统计软件进行分析，结果中组间均数比较采用t检验，率的比较采用χ2检验。P<0.05表示差异有统计学意义。

2 结果

2.1 患者的基本数据

共纳入患者453例，FET周期453个。患者平均年龄33.5±0.47岁，平均不孕年限(4.4±3.3)年。患者的平均年龄、不孕年限、移植日内膜厚度等基本资料和激光切片组与对照组间均无统计学差异(P>0.05)，详见表1。

2.2 激光切片法对分裂期FET的影响

为了探讨激光切片法对分裂期FET妊娠结局的影响，我们共选取了37个玻璃化冷冻移植周期进行分析。其中行激光切片组169个周期，移植胚胎328
Table 1 General information of patients (x̄ ± s, %)

<table>
<thead>
<tr>
<th>指标</th>
<th>激光削薄组</th>
<th>对照组</th>
</tr>
</thead>
<tbody>
<tr>
<td>周期数(n)</td>
<td>212</td>
<td>241</td>
</tr>
<tr>
<td>年龄(岁) Age (year)</td>
<td>32.9 ± 5.0</td>
<td>33.6 ± 4.9</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>21.49 ± 2.82</td>
<td>21.36 ± 2.45</td>
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<tr>
<td>不孕年限(a)</td>
<td>4.4 ± 3.2</td>
<td>4.3 ± 3.3</td>
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<td>Infertility duration</td>
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<tr>
<td>既往移植失败次数(n)</td>
<td>1.3 ± 1.8</td>
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<tr>
<td>Times of previous transplant failures</td>
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<tr>
<td>不孕原因 Causes of infertility (%)</td>
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<tr>
<td>女方 Female</td>
<td>66.98 (142/212)</td>
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<tr>
<td>男方 Male</td>
<td>12.26 (26/212)</td>
<td>7.88 (19/241)</td>
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<td>混合 Mix</td>
<td>20.75 (44/212)</td>
<td>22.41 (54/241)</td>
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<td>不孕类型 Type of infertility</td>
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<tr>
<td>原发不孕</td>
<td>50.00 (106/212)</td>
<td>46.47 (112/241)</td>
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<td>Primary infertility</td>
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<td>继发不孕</td>
<td>50.00 (106/212)</td>
<td>53.53 (129/241)</td>
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<tr>
<td>Secondary infertility</td>
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<tr>
<td>授精方式 Insemination (%)</td>
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<tr>
<td>IVF</td>
<td>74.06 (157/212)</td>
<td>76.35 (184/241)</td>
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<tr>
<td>ICSI</td>
<td>24.53 (52/212)</td>
<td>21.58 (52/241)</td>
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<tr>
<td>IVF/ICSI</td>
<td>1.42 (3/212)</td>
<td>2.07 (5/241)</td>
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<tr>
<td>内膜准备方案 Endometrial preparation (%)</td>
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</tr>
<tr>
<td>自然周期</td>
<td>37.26 (79/212)</td>
<td>33.20 (80/241)</td>
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<tr>
<td>Natural cycle</td>
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<tr>
<td>激素替代</td>
<td>27.83 (59/212)</td>
<td>31.54 (76/241)</td>
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<td>Hormone replacement</td>
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<td>促排卵</td>
<td>34.91 (74/212)</td>
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<td>Ovulation</td>
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<td>移植日内膜厚度(mm)</td>
<td>11.0 ± 2.5</td>
<td>11.1 ± 2.7</td>
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<td>Endometrial thickness on transfer day</td>
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<td></td>
</tr>
<tr>
<td>胚胎复苏率(%)</td>
<td>98.50 (394/400)</td>
<td>97.65 (458/469)</td>
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<tr>
<td>Embryo survival rate</td>
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<td></td>
</tr>
<tr>
<td>平均移植胚胎数(n)</td>
<td>1.9 ± 0.4</td>
<td>1.9 ± 0.4</td>
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<tr>
<td>Average number of embryos transferred</td>
<td></td>
<td></td>
</tr>
<tr>
<td>生化妊娠率(%)</td>
<td>50.47 (107/212)</td>
<td>50.62 (122/241)</td>
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<tr>
<td>Biochemical pregnancy rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>临床妊娠率(%)</td>
<td>43.40 (92/212)</td>
<td>44.40 (107/241)</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>胚胎种植率(%)</td>
<td>30.20 (119/394)</td>
<td>29.91 (137/458)</td>
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<tr>
<td>Implantation rate</td>
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<tr>
<td>多胎率(%)</td>
<td>30.43 (28/92)</td>
<td>28.04 (30/107)</td>
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<tr>
<td>Multiple pregnancy rate</td>
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<td></td>
</tr>
<tr>
<td>活产率(%)</td>
<td>35.85 (76/212)</td>
<td>37.34 (90/241)</td>
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<tr>
<td>Live birth rate</td>
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<td></td>
</tr>
<tr>
<td>流产率(%)</td>
<td>17.39 (16/92)</td>
<td>15.89 (17/107)</td>
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<tr>
<td>Abortion rate</td>
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</table>

Table 2 Effect of laser thinning on the cleavage stage frozen-thawed embryo transfer (x̄ ± s, %)

<table>
<thead>
<tr>
<th>指标</th>
<th>激光削薄组</th>
<th>对照组</th>
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<tr>
<td>周期数(n)</td>
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<td>203</td>
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<tr>
<td>年龄(岁) Age (year)</td>
<td>33.0 ± 5.1</td>
<td>33.7 ± 4.8</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>21.73 ± 2.92</td>
<td>21.43 ± 2.51</td>
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<td>不孕年限(a)</td>
<td>4.5 ± 3.2</td>
<td>4.3 ± 3.2</td>
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<td>Infertility duration</td>
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<td></td>
</tr>
<tr>
<td>移植日内膜厚度(mm)</td>
<td>10.9 ± 2.5</td>
<td>11.2 ± 2.7</td>
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<tr>
<td>Endometrial thickness on transfer day</td>
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<td></td>
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<tr>
<td>胚胎复苏率(%)</td>
<td>99.09 (328/331)</td>
<td>97.98 (388/396)</td>
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<tr>
<td>Embryo survival rate</td>
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</tr>
<tr>
<td>平均移植胚胎数(n)</td>
<td>1.9 ± 0.3</td>
<td>1.9 ± 0.3</td>
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<tr>
<td>Average number of embryos transferred</td>
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<td></td>
</tr>
<tr>
<td>生化妊娠率(%)</td>
<td>49.11 (83/169)</td>
<td>48.28 (98/203)</td>
</tr>
<tr>
<td>Biochemical pregnancy rate</td>
<td></td>
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<tr>
<td>临床妊娠率(%)</td>
<td>42.01 (71/169)</td>
<td>43.26 (86/203)</td>
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<tr>
<td>Clinical pregnancy rate</td>
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<td></td>
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<tr>
<td>胚胎种植率(%)</td>
<td>28.66 (94/328)</td>
<td>28.35 (110/388)</td>
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<tr>
<td>Implantation rate</td>
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<td></td>
</tr>
<tr>
<td>多胎率(%)</td>
<td>33.80 (24/71)</td>
<td>27.91 (24/86)</td>
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<tr>
<td>Multiple pregnancy rate</td>
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<td></td>
</tr>
<tr>
<td>活产率(%)</td>
<td>34.91 (59/169)</td>
<td>34.48 (70/203)</td>
</tr>
<tr>
<td>Live birth rate</td>
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<td></td>
</tr>
<tr>
<td>流产率(%)</td>
<td>16.90 (12/71)</td>
<td>18.60 (16/86)</td>
</tr>
<tr>
<td>Abortion rate</td>
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<td></td>
</tr>
</tbody>
</table>
表 3 激光削薄对冻融囊胚移植的影响( x ± s , % )
Table 3 Effect of laser thinning on the frozen-thawed blastocyst transfer

<table>
<thead>
<tr>
<th>指标</th>
<th>激光削薄组</th>
<th>对照组</th>
</tr>
</thead>
<tbody>
<tr>
<td>年龄(岁)</td>
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<td>33.2 ± 5.6</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>20.49 ± 2.09</td>
<td>20.98 ± 2.06</td>
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<tr>
<td>不孕年限(年)</td>
<td>4.3 ± 3.1</td>
<td>4.4 ± 4.1</td>
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<tr>
<td>Endometrial thickness on transfer day</td>
<td>11.40 ± 2.31</td>
<td>10.50 ± 2.79</td>
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<td>平均移植胚胎数(n)</td>
<td>1.5 ± 0.6</td>
<td>1.8 ± 0.4</td>
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<tr>
<td>Average number of embryos transferred</td>
<td>60.47 (26/43)</td>
<td>63.16 (24/38)</td>
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<tr>
<td>Biochemical pregnancy rate</td>
<td>48.84 (21/43)</td>
<td>55.26 (21/38)</td>
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<tr>
<td>Clinical pregnancy rate</td>
<td>37.88 (25/66)</td>
<td>38.57 (27/70)</td>
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<tr>
<td>Implantation rate</td>
<td>19.05 (4/21)</td>
<td>28.57 (6/21)</td>
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<tr>
<td>Multiple pregnancy rate</td>
<td>39.53 (17/43)</td>
<td>52.63 (20/38)</td>
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<tr>
<td>Live birth rate</td>
<td>19.05 (4/21)</td>
<td>4.76 (1/21)</td>
</tr>
<tr>
<td>Abortion rate*</td>
<td>P&lt;0.05, 与对照组比较 with the control</td>
<td></td>
</tr>
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</table>

2.4 激光削薄法对反复移植失败患者妊娠结局的影响

为了探讨激光削薄法对反复移植失败患者妊娠结局的影响，我们共选取了既往有移植失败≥2次的患者的128个周期进行分析，其中行激光削薄组60个周期，移植胚胎107枚；对照组68个移植周期，移植胚胎130枚。结果显示，激光削薄组和对照组在年龄、不孕年限、移植日内膜厚度、生化妊娠率、临床妊娠率、胚胎种植率、活产率等多个指标上均无统计学差异(P>0.05)，但是组间的活产率存在统计学差异(P=0.035)，详见表4。

3 讨论

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表 4 激光削薄法对反复移植失败患者FET的影响
Table 4 Effect of laser thinning on the frozen-thawed embryo transfer cycles of repeated implantation failure patients

<table>
<thead>
<tr>
<th>指标</th>
<th>激光削薄组</th>
<th>对照组</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>60</td>
<td>68</td>
</tr>
<tr>
<td>年龄(岁) Age (year)</td>
<td>35.3 ± 5.2</td>
<td>35.3 ± 4.7</td>
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<td>BMI (kg/m²)</td>
<td>21.15 ± 2.55</td>
<td>21.69 ± 2.36</td>
</tr>
<tr>
<td>不孕年限(年)</td>
<td>5.3 ± 3.9</td>
<td>4.7 ± 3.6</td>
</tr>
<tr>
<td>Endometrial thickness on transfer day</td>
<td>9.86 ± 2.28</td>
<td>10.57 ± 2.47</td>
</tr>
<tr>
<td>平均移植胚胎数</td>
<td>1.8 ± 0.5</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Average number of embryos transferred</td>
<td>43.33 (26/60)</td>
<td>45.59 (31/68)</td>
</tr>
<tr>
<td>Biochemical pregnancy rate</td>
<td>36.67 (22/60)</td>
<td>39.71 (27/68)</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>25.23 (27/107)</td>
<td>26.15 (34/130)</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>22.73 (5/22)</td>
<td>25.93 (7/27)</td>
</tr>
<tr>
<td>Multiple pregnancy rate</td>
<td>23.33 (14/60)</td>
<td>35.29 (24/68)</td>
</tr>
<tr>
<td>Live birth rate</td>
<td>36.36 (8/22)</td>
<td>11.11 (3/27)</td>
</tr>
<tr>
<td>Abortion rate*</td>
<td>P&lt;0.05, 与对照组比较 with the control</td>
<td></td>
</tr>
</tbody>
</table>
者提出需要在FET前对其进行AH操作。利用胚胎的顺利孵出和着床。但是，现有的研究结果对此却存在着重大的争议。多数学者认为[25,26], AH技术能够明显改善FET患者的妊娠结局。然而也有研究者认为[27-29], AH技术并不能明显改善FET患者的妊娠结局，甚至可能会降低FET患者的胚胎种植率。在本研究中，我们分别以玻璃化冷冻的分裂期和囊胚期胚胎为对象进行激光削薄AH效果研究。结果显示，分裂期胚胎激光削薄组患者的生化妊娠率、临床妊娠率及胚胎种植率与对照组相比均无统计学差异；囊胚期胚胎患者的移植结局与对照组相比，其生化妊娠率、临床妊娠率及胚胎种植率均无统计学差异，结果与Petersen及Ng等学者报道的研究结果[27,28]一致。此外，我们还观察到对反复移植失败患者行AH并不能明显改善其妊娠结局，激光削薄组患者的生化妊娠率、临床妊娠率及胚胎种植率与对照组无统计学差异。这些研究结果提示，对玻璃化冷冻胚胎进行激光削薄AH并不能有效改善移植患者的妊娠结局。因此我们不提倡对冷冻胚胎进行常规化的AH操作。

激光AH技术是利用激光的热效应对透明带造成气化作用，从而使透明带变薄，但是激光的热效应是否会对胚胎造成不良作用，从而影响胚胎的进一步发育，目前相关研究报道不多。Germond等利用透射电子显微镜观察由激光引起的邻近细胞的改变[30]，认为激光AH技术对胚胎的超微结构和生物学状态无显著影响。提示激光法可能是相对安全的辅助生殖技术。在本研究中，患者异位妊娠发生比例低。仅在分裂期胚胎的激光削薄组中发生1例。其余各组患者均未发生。提示激光削薄AH技术可能与异位妊娠的发生无明确相关性，与Jun等的研究结果[210]相反。

但是，我们的研究结果还表明，在囊胚期胚胎移植和反复移植失败患者中，激光削薄组患者移植后的活产率较对照组有增高的趋势。其原因可能是激光的热效应对移植前胚胎造成了显著的不良影响，从而降低了胚胎后期发育潜能，这一推测还需扩大样本进一步验证。综上所述，我们的研究认为，激光削薄AH技术并不能明显改善冻融胚胎移植患者的妊娠结局，其远期安全性尚需继续追踪研究。临床工作中对于FET前进行激光AH应持谨慎态度。

参考文献：

Effect of Laser Thinning Assisted Hatching on Vitrified-warmed Embryo Transfer

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**Abstract**

**Objective:** To explore the effect of laser thinning assisted hatching on frozen-thawed embryo transfer outcome. 
**Methods:** Selected embryos from 372 cleavage stage embryo transfer cycles, 81 blastocyst transfer cycles and 128 repeated implantation failure cycles were processed, and divided into laser thinning group and control group respectively according to odd-numbered days and even-numbered days of embryo thawed day.
The embryos in laser thinning group received laser thinning assisted hatching (AH) treatment before fertilization, and which in control group did not. The laboratory and clinical outcomes were compared between the two groups.

**Results:** The biochemical pregnancy rate, the clinical pregnancy rate and the implantation rate in the assisted hatching cleavage stage embryo group showed no significant differences with those of the control (49.11% vs 48.28%, 42.01% vs 42.36%, 28.66% vs 28.35%, P>0.05); There was no statistical difference of biochemical pregnancy rate, clinical pregnancy rate and implantation rate between the assisted hatching blastocyst group and the control group (60.47% vs 63.16%, 48.84% vs 55.26%, 37.88% vs 38.57%, P>0.05); the application of laser assisted hatching did not improve the pregnancy outcomes of repeated implantation failure patients (43.33% vs 45.59%, 36.67% vs 39.71%, 25.23% vs 26.15%, P>0.05). However, in the blastocyst transfer patients and repeated implantation failure patients, there was an increasing trend of abortion rate in laser assisted hatching group (19.05% vs 4.76%, P=0.153; 36.36% vs 11.11%, P=0.035). **Conclusion:** The laser assisted hatching technique does not improve pregnancy outcomes of frozen-thawed embryo transfer patients, and its long-term safety needs further study. The clinical application of this method should be cautious.

**Key words:** laser; assisted hatching (AH); vitrification freezing; embryo transfer (ET)

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**A Dynamic Method of Embryo Culture Based on A Microfluidic Chip**

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**[ABSTRACT] Objective:** To develop a microfluidic chip for embryo dynamic culture method nearly physiological status. **Methods:** A microfluidic chip was designed and fabricated for testing the influence of continual fluid flow on embryo development. This gave rise to design of a microfluidics system using microchannels as conduits for fluid flow through a 16-microcellular where mouse embryos resided by mimicking the fluid-mechanical and biochemical stimulation embryos experience in vivo from ciliary currents and oviductal contractions. And compared with conventional droplet culture method, monitoring of the embryonic development conditions and blastocyst formation rate. **Results:** The microfluidic chip dynamic culture method can significantly improve the embryo development. The microfluidic dynamic method was superior to the microdrop-static method in terms of 4-cell embryo rate (68.4 ± 1.2% vs 53.2 ± 2.5%), morula rate (55.3 ± 2.6% vs 45.5 ± 3.3%) and blastocyst rate (45.5 ± 2.7% vs 35.5 ± 2.3%)(P<0.05). These two methods didn’t show significant difference in the rate of 2-cell embryos (75.5 ± 3.2% vs 73.9 ± 4.2%, P>0.05). **Conclusion:** Physiological embryo culture was achieved on a microfluidic chip. This microfluidic method was able to improve embryo development and showed advantage over the conventional method, which was expected to serve as a powerful tool for embryo culture in the future.

**Key words:** microfluidic chip; dynamic culture; embryo development