

• 实验研究 •

## 小鼠睾丸支持细胞标志蛋白表达的研究

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**【摘要】**目的: 探寻小鼠睾丸支持细胞不同发育阶段和功能状态下的特异性标志蛋白。方法: 通过对小鼠睾丸组织进行免疫组织化学染色和实时定量(real-time)RT-PCR, 观察受精后16 d胚胎(E16)到出生后6周的雄性小鼠睾丸组织蛋白和mRNA表达。结果: 雄激素受体(AR)在出生后1周、2周、6周小鼠的睾丸支持细胞、外周小管肌样细胞、间质细胞细胞核内都有表达, 且表达量逐渐增强, 但在E16不表达; Wilms 肿瘤基因(WT1)在E16、出生后1周、2周、6周的睾丸支持细胞细胞核内表达, 但不表达于其它细胞; 苗勒氏管抑制素(AMH)在E16、出生后2周的睾丸支持细胞胞质中表达, 而在出生后6周不表达;  $\beta$ -链蛋白( $\beta$ -catenin)和胞质紧密粘连蛋白-1(Zo-1)是血睾屏障特异性蛋白, 出生后2周时它们分布于整个曲细精管, 而出生后4周时规则地分布于靠近外壁的一圈, 此时它们的血睾屏障已经完全建立。Real-time PCR 分析睾丸组织 mRNA 表达, 佐证了这些结果。结论: WT1可以作为支持细胞的特异性标志, 而AMH则标记不成熟的睾丸支持细胞; AR单独不适合作为睾丸支持细胞的标志物, 但与其他标志物结合, 可能可区分睾丸支持细胞的成熟与否。而通过 $\beta$ -catenin和Zo-1染色, 可以判断血睾屏障是否完全形成, 为支持细胞是否成熟提供判断标记。

**关键词:** 睾丸; 支持细胞; 标志蛋白; 小鼠

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## Investigations on the expression of protein markers in Sertoli cells

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**【ABSTRACT】 Objective:** To examine whether some markers are regarded as accuracy label of Sertoli cells at different development stages, function status of Sertoli cells. **Methods:** By immunohistochemical staining and real-time PCR methods, the expression of Sertoli cell marker in testis were examined from embryo period 16 d (E16) to 6 weeks after birth. **Results:** Protein expression of androgen receptor (AR) was observed in nucleus of Sertoli cells, peritubular myoid cells and Leydig cells in 1 week, 2-week, 6-week old mice. Protein expression of Wilms tumour gene 1 (WT1) was observed in Sertoli cells' nucleus in E16 and 1 week, 2 weeks, 6 weeks after birth. Protein expression of anti-Müllerian hormone (AMH) was observed in Sertoli cells' cytoplasm at E16-2 weeks after birth. Until postnatal 4-week blood testis barrier (BTB) complex protein  $\beta$ -catenin, Zonula occludens-1 (ZO-1) only expressed near basement memberane of seminiferous tubule. By analysis of mRNA expression of testis, these results were confirmed. **Conclusion:** WT1 could be regarded as protein marker of Sertoli cells at all the development stages. AMH could be regarded as an immature protein marker of Sertoli cells. AR could not be regarded as protein marker of Sertoli cells, but could be a mature marker of Sertoli cells if it is co-immunofluorescence stained with another Sertoli cell marker. By staining  $\beta$ -catenin or ZO-1, we could judge whether or not Sertoli cells create BTB fully and Sertoli cells is mature.

**Key words:** testis; Sertoli cells; protein marker; mouse

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# 坤泰胶囊对大鼠实验性子宫异位内膜生长及其妊娠结局的影响

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**【摘要】**目的: 研究坤泰胶囊(KTC)对SD大鼠实验性子宫异位内膜生长及其妊娠结局的影响。方法: 手术法建立SD大鼠子宫内膜异位症(EMs)模型。将造模成功的SD大鼠分为模型对照组、阳性对照组(每只给予醋酸亮丙瑞林0.1 mg皮下注射, 共1次), KTC低剂量组(每只按0.24 g/kg体质量灌胃, qd × 21 d)、KTC高剂量组(每只按0.60 g/kg体质量灌胃, qd × 21 d), 每组14只。给药21 d后, 每组解剖8只, 比较给药前、后异位内膜体积变化; Western blotting法测定异位内膜caspase-8、caspase-3和caspase-9蛋白表达。每组剩余6只与雄鼠交配, 妊娠12 d后, 解剖观察受孕情况, 并对胎盘、胎仔称量。动情周期正常的SD大鼠14只作为空白对照。结果: 与模型对照组相比, KTC低、高剂量组体质量无统计学差异( $P>0.05$ ); KTC低、高剂量组异位内膜抑制率分别为(40.25 ± 35.18)%和(65.52 ± 15.82)%; KTC低、高剂量组异位内膜中caspase-8、caspase-9和活化caspase-3明显升高( $P<0.05$ ); KTC低剂量组的大鼠胎仔数量呈增加趋势。结论: KTC通过升高caspase-8、caspase-9和活化caspase-3蛋白含量, 促进异位内膜的凋亡; KTC低剂量组EMs大鼠受孕率呈现出升高的趋势。

**关键词:** 坤泰胶囊(KTC); 子宫内膜异位症(EMs); SD大鼠; caspase; 妊娠率

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## Effect of Kuntai capsule on ectopic endometrium and the pregnancy outcome in a rat of endometriosis

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**【ABSTRACT】 Objective:** To investigate the effect of Kuntai capsule (KTC) on endometriosis (EMs) the growth of ectopic endometrium and the pregnancy outcome in SD rats. **Methods:** Rat model of EMs was established with surgically implanted endometrium, and then the animals were given different doses of KTC in 4 groups of 14 rats each. After 3 weeks of treatment, laparotomy was performed to examine the volume changes in the ectopic endometrium for 8 rats per group. Western blotting assay was used to test the protein level of caspase-8, caspase-9 and caspase-3 in ectopic endometrium. The remaining 6 rats per group were mated with male rats. After successful pregnancy for 12 d, the fetal rats and placenta were weighed. **Results:** Compared with the model group, the inhibitory rates were  $(40.25 \pm 35.18)\%$  and  $(65.52 \pm 15.82)\%$ , respectively in the group of KTC 0.24 g/kg and 0.60 g/kg, qd  $\times$  21 d; KTC induced apoptosis in ectopic endometrium, through increasing the protein expression of caspases-8 and caspases-9, activated caspase-3. The pregnancy rate increased by 67% and the average number of fetus increased by 2 times in KTC low-dose group compared with the model group. **Conclusion:** The KTC inhibited the growth of ectopic endometrial tissue via improving the expression of caspase-8, caspases-9, activated caspase-3, and the pregnancy rate was improved in the group of KTC 0.24 g/kg treatment.

**Key words:** Kuntai capsule (KTC); endometriosis (EMs); SD rat; caspase; pregnancy rate

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# 人生长分化因子-9(*GDF-9*)基因真核表达载体的构建及表达

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**【摘要】**目的: 构建人生长分化因子-9(*GDF-9*)基因真核表达载体并分离纯化获得重组 *GDF-9* 蛋白。方法: 以 cDNA 克隆为模板, PCR 法扩增出人 *GDF-9* 基因, 将目的基因连接到 pcDNA3.1(+)真核表达载体, 构建重组质粒 pcDNA3.1(+)/*GDF-9*, 脂质体法将重组质粒转染到 HEK293T 细胞, 通过 Western blotting 检测 *GDF-9* 蛋白表达。结果: 以双酶切及基因测序鉴定构建的重组真核质粒, Western blotting 方法证实人 *GDF-9* 基因正确表达。结论: 成功构建人 *GDF-9* 基因 pcDNA3.1(+)/*GDF-9* 重组质粒, 并在真核细胞系表达, 获得重组 *GDF-9* 蛋白, 为进一步研究 *GDF-9* 基因提供便利工具。

**关键词:** 卵源性因子; 生长分化因子-9(*GDF-9*); 重组表达; HEK293T 细胞

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## Construction and expression of the eukaryotic expression vector of human growth differentiation factor-9 (*GDF-9*) gene

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**【ABSTRACT】 Objective:** To construct the eukaryotic expression vector of human growth differentiation factor-9 (*GDF-9*) gene and obtain purified recombinant *GDF-9* protein. **Methods:** Human *GDF-9* gene was cloned by PCR method using the complementary DNA, and was inserted into pcDNA3.1(+) vector to construct recombinant eukaryotic expression plasmid pcDNA3.1(+)/*GDF-9*, which was transfected into human embryonic kidney (HEK) 293T cell line by liposome transfecting technique. The expression of *GDF-9* was detected by Western blotting. **Results:** Recombinant plasmid constructed successfully was confirmed by double enzyme digestion and gene sequencing result. Recombinant protein *GDF-9* successfully was verified by Western blotting. **Conclusion:** The recombinant eukaryotic expression plasmid pcDNA3.1 (+)/*GDF-9* was successfully constructed. Recombinant protein *GDF-9* was successfully obtained, which is conducive for the further research of the function of *GDF-9*.

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• 临床研究 •

## 应用时差成像技术挑选优质胚胎改善 单胚胎移植妊娠结局

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**【摘要】**目的: 探讨应用时差成像(TLI)技术基于胚胎卵裂模式及时间参数进行胚胎挑选, 对分裂期胚胎单胚胎移植(SET)妊娠结局的影响。方法: ①将206个常规IVF周期患者胚胎行TLI动态观察, 对其2349枚非移植胚胎进行囊胚培养, 回顾性分析胚胎发育的卵裂模式和时间参数, 以囊胚质量为结局进行Logistic回归分析, 建立优质胚胎筛选标准。②应用建立的优质胚胎筛选标准开展分裂期胚胎SET的前瞻性研究, 用TLI观察88例常规IVF周期患者的胚胎发育情况, 挑选优质胚胎进行SET, 观察妊娠结局。结果: ①分裂方式为正常卵裂、不均卵裂、非轴性卵裂的胚胎具有较高的优质囊胚形成率(分别为57.09%, 44.44%及40.91%), 时间参数从3-细胞分裂到4-细胞持续的时间( $s_2$ ) ( $\leq 0.50$  h)和发育至5-细胞的时间( $t_5$ ) ( $\leq 48.93$  h)对优质囊胚形成最具预测价值。②由此建立基于胚胎卵裂模式与时间参数的分层筛选模式, 将其应用于分裂期胚胎SET, 符合筛选条件的SET组临床妊娠率为73.24%(52/71)。结论: 应用TLI, 基于胚胎卵裂模式和时间参数建立了分层筛选模式, 所挑选的优质胚胎可显著改善分裂期胚胎SET的妊娠结局。

**关键词:** 单胚胎移植(SET); 时差成像(TLI); 卵裂模式; 时间参数

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• 临床研究 •

## Selection of high-quality embryos by time-lapse imaging for improving the pregnancy outcome of single embryo transfer

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**【ABSTRACT】 Objective:** To discuss whether the selection of embryos based on embryo cleavage patterns and time parameters by time-lapse imaging (TLI) can improve the pregnancy outcome of single embryo transfer (SET) in the cleavage stage. **Methods:** A retrospective analysis on the data of 206 patients with IVF cycles was performed by TLI dynamic observation, of which a total of 2 349 non-transplanted embryos were used for blastocyst culture. A Logistic regression analysis on time parameters and cleavage patterns of embryo development was conducted by taking the blastocyst quality as the outcome and a good-quality embryo screening standard was established. A prospective study on SET was carried out in the cleavage stage by application of this standard. The embryo development of 88 patients with traditional IVF cycle was observed by TLI, the SET was done by selecting high-quality embryos, and the pregnancy outcomes were observed. **Results:** The Logistic regression analysis showed embryos with normal cleavage, uneven cleavage and non-axial cleavage had a high formation rate of good-quality blastocyst (57.09%, 44.44% and 40.91%, respectively).  $S_2$  ( $\leq 0.50$  h) and  $t_5$  ( $\leq 48.93$  h) were the time parameters with the most predictive value for formation of high-quality blastocysts. A hierarchical screening mode based on embryo cleavage patterns and time parameters was established and was used for SET in the cleavage stage, and the clinical pregnancy rate of SET in line with the screening conditions was 73.24% (52/71). **Conclusion:** The hierarchical screening mode established based on the embryo cleavage patterns and time parameters can significantly improve the clinical pregnancy rate of SET in the cleavage stage by selecting high-quality embryos.

**Key words:** single embryo transfer (SET); time-lapse imaging (TLI); cleavage pattern; timing parameter



# 诱导骨髓SSEA-1<sup>+</sup>干细胞向卵母细胞分化的初步研究

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**【摘要】**目的: 探讨骨髓SSEA-1<sup>+</sup>干细胞向卵母细胞分化的潜能。方法: 采用免疫磁珠分选系统(MACS)分选骨髓中SSEA-1<sup>+</sup>干细胞, 接种于小鼠成纤维细胞(mEFs)饲养层上, 在含1 000 U/ml 白血病抑制因子(LIF)的DMEM/F12培养液中将SSEA-1<sup>+</sup>干细胞传代培养至第2代(P2), 撤去饲养层, 在含20 ng/ml 骨形成蛋白4 (BMP4)的培养液中进行定向诱导, 并分阶段加入体积分数10%人卵泡液、0.005 IU/ml 卵泡刺激素(FSH)和0.003 IU/ml 黄体生成素(LH)。光学显微镜下观察细胞形态学变化, 检测不同发育阶段生殖细胞相关特异性基因*Dazl*、*Fragilis*、*Mvh*、*Nobox*、*Oct4*、*Stella*、*STRA8*、*Gdf9*的表达。结果: 骨髓SSEA-1<sup>+</sup>干细胞生殖细胞相关特异性基因表达水平均显著高于骨髓单个核细胞和SSEA-1<sup>-</sup>细胞( $P < 0.05$ )。小鼠骨髓SSEA-1<sup>+</sup>干细胞与mEFs共培养后呈圆形, 碱性磷酸酶染色阳性, 显示细胞处于未分化状态。BMP4诱导7 d SSEA-1<sup>+</sup>干细胞形成细胞集落, 14 d可见类胚体(EB)样结构形成, 21 d可观察到卵母细胞样结构, 且随诱导时间的延长生殖细胞相关特异性基因尤其卵母细胞减数分裂基因*Stra8*和*Gdf9*表达水平显著升高。结论: 骨髓SSEA-1<sup>+</sup>干细胞具备向类卵母细胞样细胞分化的潜能。

**关键词:** 骨髓间充质干细胞(BMSCs); 卵母细胞样细胞; 骨形成蛋白4 (BMP4); 细胞分化; qRT-PCR

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## Derivation of oocyte-like cells from SSEA-1<sup>+</sup> stem cells of mouse bone marrow

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**【ABSTRACT】 Objective:** To explore the derivative potential of stage specific embryonic antigen (SSEA)-1<sup>+</sup> stem cells of mouse bone marrow toward oocyte-like cells *in vitro*. **Methods:** SSEA-1<sup>+</sup> stem cells were separated from mouse bone marrow by magnetic activated cell sorting (MACS), and cultured on mouse embryonic fibroblasts feeder layer (mEFs) in DMEM/F12 medium contained leukemia inhibitory factor (LIF). The Passage 2 (P2) SSEA-1<sup>+</sup> cells were removed from mEFs and induced to differentiate toward germ line under 20 ng/ml bone morphogenetic protein (BMP)4, then 10% human follicular fluid, 0.005 IU/ml follicle stimulating hormone (FSH) and 0.003 IU/ml luteinizing hormone (LH) were added to the medium at different differentiation stages. Meanwhile, the morphological observation and detection of early germ cell-specific genes related to germ cell differentiation such as *Dazl*, *Fragilis*, *Mvh*, *Nobox*, *Oct4*, *Stella*, *STRA8* and *Gdf9* were performed at different differentiation stages. **Results:** The expression levels of all selected early germ cell-specific genes were significantly higher in SSEA-1<sup>+</sup> stem cells than those in whole bone marrow cells and SSEA-1<sup>-</sup> cells ( $P < 0.05$ ). Mouse bone marrow SSEA-1<sup>+</sup> stem cells grew well and maintained undifferentiated state on mEFs feeder, with roughly circle shapes and positive alkaline phosphatase staining. The cell colonies, embryoid body (EB) structures, oocyte-like cells from P2 SSEA-1<sup>+</sup> stem cells could be seen on day 7, 14 and 21 respectively after induction of BMP4, and the expression levels of germ cell specific genes were increased gradually, especially for *STRA8* and *Gdf9*. **Conclusion:** Under the induction of BMP4, SSEA-1<sup>+</sup> bone marrow mesenchymal stem cells could differentiate into oocyte-like cells *in vitro*.

**Key words:** bone marrow mesenchymal stem cells (BMSCs); oocytes; bone morphogenetic protein (BMP) 4; cell differentiation; qRT-PCR

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# 盆底型子宫内膜异位症患者行IVF-ET助孕后妊娠结局及妊娠期并发症的分析

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**【摘要】**目的: 探讨盆底型子宫内膜异位症(EMs)患者行IVF-ET助孕后妊娠结局及妊娠期并发症。方法: 回顾性分析行IVF-ET治疗获临床妊娠的盆底型EMs 96个周期(A组), 卵巢子宫内膜异位囊肿者 107个周期(B组), 并选择同期 180个周期输卵管因素行IVF-ET助孕获临床妊娠者为对照组(C组)。分析比较各组患者行IVF-ET助孕的妊娠结局及妊娠期并发症。结果: A组早产率显著低于B组(9.38% vs 21.50%,  $P < 0.05$ ); 流产率(25.00% vs 12.78%)及单胎流产率(35.00% vs 17.31%)明显高于C组, 差异有统计学意义( $P < 0.05$ ); 异位妊娠发生率略高于B组(2.08% vs 1.87%), 低于C组(6.67%), 但3组间无统计学差异( $P = 0.072$ )。A组和B组妊娠期并发症发生率明显高于C组(30.21% vs 31.78% vs 16.11%,  $P < 0.05$ ), 但A、B组间无统计学差异( $P > 0.05$ )。其中A组和B组子痫前期(8.33% vs 9.35%)、前置胎盘(9.38% vs 10.28%)发生率显著高于C组(2.78%; 3.33%); A组先兆流产率高于B组和C组(18.75% vs 14.02% vs 8.33%), 且与C组差异有统计学意义( $P < 0.05$ )。结论: 盆底型EMs患者行IVF-ET助孕其自然流产率显著高于输卵管不孕患者, 早产率较卵巢型EMs降低; 妊娠期并发症较输卵管不孕患者明显增多, 主要表现在子痫前期、前置胎盘及先兆流产3个方面, 而与卵巢型EMs无统计学差异。

**关键词:** 体外受精 - 胚胎移植(IVF-ET); 盆底型子宫内膜异位症(EMs); 妊娠结局; 妊娠期并发症

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## Pregnancy complications and outcomes in patients with pelvic floor endometriosis after *in vitro* fertilization-embryo transfer

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**【ABSTRACT】 Objective:** To analyze the pregnancy complications and outcomes in patients with pelvic floor endometriosis (EMs) after IVF-ET. **Methods:** A retrospective study was conducted patients who underwent IVF-ET. The pregnancy complications and outcomes were compared among 96 cycles of pelvic floor EMs (group A), 107 cycles of ovarian EMs (group B) and randomly chosen 180 cycles of tube factor infertility (group C). **Results:** Incidence of preterm birth was significantly lower in group A than in group B (9.38% vs 21.50%,  $P<0.05$ ). The incidence of miscarriage (25.00% vs 12.78%) and single birth miscarriage (35.00% vs 17.31%) were significantly higher in group A than in group C. The incidence of ectopic pregnancy in group A was higher than that in group B (2.08% vs 1.87%) and lower than that in group C (2.08% vs 6.67%), but there were no significant differences among three groups ( $P=0.072$ ). The incidence of pregnancy complications in groups A and B was significantly higher than that in group C (30.21% vs 31.78% vs 16.11%,  $P<0.05$ ), but there was no significant difference between groups A and B ( $P>0.05$ ). The rates of preeclampsia (8.33% vs 9.35% vs 2.78%) and placenta previa (9.38% vs 10.28% vs 3.33%) in groups A and B were significantly higher than those in group C. The rate of threatend abortion in group A was higher than that in groups B and C, which had a significant difference when compared with group C (18.75% vs 14.02% vs 8.33%). **Conclusion:** The patients with pelvic floor EMs after IVF-ET have significant higher spontaneous miscarriage rate than tube factor infertilities and lower preterm birth rate than that of ovarian EMs patients. The pregnancy complications are higher than tube factor infertilities mainly in preeclampsia, placenta previa and threatend abortion, but there are no significant differences when compared with ovarian EMs patients.

**Key words:** *in vitro* fertilization-embryo transfer (IVF-ET); pelvic floor endometriosis (EMs); pregnancy outcomes; pregnancy complications

# 年轻女性首次体外受精长方案周期 累积活产率相关因素分析

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**【摘要】**目的: 探讨年轻女性体外受精-胚胎移植(IVF-ET)长方案周期累积活产率的影响因素。方法: 回顾性分析首次接受IVF长方案助孕治疗的1 147例年轻且卵巢储备功能良好的不孕女性的临床资料, 按完成一次取卵周期是否获得最终活产胎儿分为活产组与未获活产组, 采用多因素Logistic回归分析影响累积流产率的相关因素。结果: 活产组患者的体质量指数(BMI)较未获活产组显著降低( $P<0.05$ ), 而获卵数、正常受精率及可移植胚胎数显著增高( $P<0.05$ )。Logistic回归分析显示BMI及可移植胚胎数是年轻且卵巢储备功能良好的不孕女性首次行IVF长方案周期获得累积活产的重要影响因素, BMI $<24.2$  kg/m<sup>2</sup>及可移植胚胎数 $\geq 5$ 患者的妊娠结局较佳。结论: 年轻且卵巢储备功能良好的女性首次IVF长方案周期中, BMI及可移植胚胎数与累积活产率显著相关, 较低的BMI及适当多的可移植胚胎数可提高一次取卵周期的累积活产率。

**关键词:** 体外受精-胚胎移植(IVF-ET); 长方案促排卵; 累积活产; 体质量指数(BMI); 可移植胚胎数

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## Related factors of cumulative live birth of young women treated with long protocol for the first time in IVF-ET

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**【ABSTRACT】 Objective:** To analyze the potential related factors of cumulative live birth rate with long protocol in *in vitro* fertilization and embryo transfer (IVF-ET). **Methods:** A total of 1 147 cycles were divided into two groups: obtained cumulative live birth group (group A) and without obtained cumulative live birth group (group B). The potential related factors of cumulative live birth were evaluated by Logistic regression analysis. **Results:** The average body mass index (BMI) of group A was much lower than that of group B ( $P<0.05$ ), while oocyte number, fertilization rate and the number of transferable embryos in group A were significantly higher than those in group B ( $P<0.05$ ). Logistic regression analysis showed that BMI and the number of transferable embryos were the two vital factors of cumulative live birth with long protocol treatment in young women. Furthermore,  $BMI<24.2\text{ kg/m}^2$  and the number of transferable embryos  $>5$  help young infertile women obtain better pregnancy outcomes. **Conclusion:** To young infertile women with normal ovarian reserve, BMI and the number of transferable embryos had a strong relationship with cumulative live birth in the first long treatment cycle. The decreased BMI and increased number of transferable embryo can improve the cumulative live birth rate according to one oocyte retrieval cycle.

**Key words:** *in vitro* fertilization and embryo transfer (IVF-ET); long protocol; cumulative live birth; body mass index (BMI); the number of transferable embryo

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• 综述 •

## 时差成像系统在IVF-ET的临床应用进展

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**【摘要】**时差成像系统(time-lapse imaging, TLI)作为一种新的胚胎选择体系, 越来越受到国内、外辅助生殖领域临床医生与胚胎学家的青睐。近年来, TLI应用于体外受精(*in vitro* fertilization, IVF)中的临床研究也逐渐增多。胚胎学家应用这一系统后不需将胚胎从培养箱中移出即可通过视频图像连续观察其整个动态发育过程, 以及获得预测胚胎发育潜能的动力学参数。应用TLI提供的动力学参数结合形态学选择移植胚胎, 可以改善胚胎着床率和临床妊娠率。但TLI技术无论从硬件还是软件方面均面临着巨大挑战; 另外, TLI系统的安全性亦有待进一步评估。

**关键词:** 时差成像系统(TLI); 体外受精(IVF); 临床应用

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• 综述 •

## Progress on clinical application of time-lapse imaging in IVF-ET

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**【ABSTRACT】** Time-lapse imaging (TLI), as a new system of embryo selection, is more and more popular in the field of assisted reproduction technology (ART) for clinicians and embryologists at home and abroad. In recent years, the clinical application of TLI in IVF is gradually increasing. Through applying this system, embryologists don't need to move out of the embryo from the incubator but via video image observation of its whole dynamic development process, and obtain kinetic parameters of predict embryonic development potential. The embryonic kinetic parameters combining morphological selection can improve embryo implantation rate and clinical pregnancy rate. TLI technologies from the hardware and software are facing great challenges. In addition, TLI system's safety has yet to be further assessed.

**Key words:** time-lapse imaging (TLI); *in vitro* fertilization (IVF); clinical application

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# 米非司酮终止妊娠作用机制的研究进展

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**【摘要】**米非司酮(mifepristone, RU486)是国际上第一个上市的用于终止妊娠的药物, 不仅已被临床用于终止早期和中期妊娠, 而且在终止中晚期妊娠方面也有潜在的临床应用前景。通过综述有关文献得出, 作为孕激素受体和糖皮质激素受体拮抗剂, 米非司酮影响母-胎界面局部的免疫细胞分化和功能, 蜕膜和绒毛细胞外基质重构, 母-胎界面各种细胞凋亡等生理过程, 诱发母-胎界面免疫耐受状态失衡, 作为导致妊娠终止的主要机制。另外, 米非司酮还通过影响子宫颈结缔组织分解和子宫平滑肌收缩等方式阻止妊娠。借助动物模型和临床数据, 全面解析米非司酮的作用机制是未来研究的趋势。

**关键词:** 米非司酮; 蜕膜; 绒毛; 细胞凋亡; 流产

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## Review on mechanism of termination of pregnancy by mifepristone

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**【ABSTRACT】** As the first commercial drugs in the world for termination of pregnancy, mifepristone (RU486) not only has been clinically used for termination of early and mid-term pregnancy, but also has prospects for clinical application in termination of late-term pregnancy. By reviewing recent literatures, as an progesterone and glucocorticoid receptor antagonist, mifepristone can interfere with the local immune state of maternal-fetal interfaces, result in extracellular matrix remodeling in chorionic villi and decidua, and apoptosis of cells at maternal-fetal interfaces so to break up immune tolerance state at maternal-fetal interfaces, eventually to lead to pregnancy termination as its involved key mechanism, other than its promotion effect in cervical connective tissue decomposition and uterine cervix smooth muscle contraction. With the help of animal model and clinical data, a comprehensive analysis on its mechanism will be in the trend of future research.

**Key words:** mifepristone; decidua; chorionic villus; apoptosis; abortion

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• 临床报道 •

## 体外受精新鲜胚胎移植术后14 d血清 $\beta$ -hCG 浓度与妊娠结局的关系

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**【摘要】**目的: 探讨体外受精-新鲜胚胎移植术后第14日后血清 $\beta$ -hCG浓度对临床妊娠结局的预测性。方法: 对727例移植2枚第3日胚胎后第14日测血清 $\beta$ -hCG浓度 $>7$  IU/L的生化妊娠者追踪其临床结局, 并回顾性分析正常宫内妊娠、异位妊娠、早期流产与血清 $\beta$ -hCG浓度的关系。结果: 本组生化妊娠者, 当其血清 $\beta$ -hCG在 $<45$  IU/L时, 无正常宫内妊娠; 46~100 IU/L时生化妊娠流产率为50.00%, 正常宫内妊娠的发生率为25.00%, 异位妊娠发生率为10.71%, 早期流产率为14.29%。生化妊娠流产发生率随着血清 $\beta$ -hCG水平升高而下降, 血清 $\beta$ -hCG $>200$  IU/L发生率仅为4.17%。血清 $\beta$ -hCG $>400$  IU/L者异位妊娠发生率(0.19%)与 $\leq 400$  IU/L(10.20%)相比明显下降( $P=0.007$ ); 当血清 $\beta$ -hCG $>500$  IU/L时, 早期流产率明显低于血清 $\beta$ -hCG $<500$  IU/L(4.66% vs 12.88%,  $P=0.002$ )。正常宫内妊娠血清 $\beta$ -hCG截断值为379.25 IU/L, ROC曲线下面积为0.868, 95%CI=0.828~0.907。异位妊娠者中71.43%妇女存在输卵管性不孕因素, 而早期流产者和生化妊娠流产者中存在男方因素性不孕分别占74.00%和68.75%。结论: 胚胎移植后第14日血清 $\beta$ -hCG水平是有效预测妊娠的指标。当血清 $\beta$ -hCG $\leq 45$  IU/L时继续黄体支持治疗意义不大; 血清 $\beta$ -hCG在46.00~379.25 IU/L范围内虽可继续黄体支持保胎, 但要警惕异位妊娠及早期流产的发生, 尤其是对有输卵管性不孕或男方因素性不孕的患者。

**关键词:** 体外受精-胚胎移植(IVF-ET); 血清 $\beta$ -hCG; 生化妊娠流产; 异位妊娠; 早期流产

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• 临床报道 •

## Relationship between serum $\beta$ -hCG level and pregnant outcome on day 14 after fresh embryo transfer

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**【ABSTRACT】 Objective:** To study the predictive value of serum  $\beta$ -hCG level for pregnant outcomes on day 14 after fresh embryo transfer. **Methods:** A total of 727 women, who got chemical pregnancy (serum  $\beta$ -hCG > 7 IU/L) after transferred two fresh day 3 embryos, were enrolled. The subsequent pregnant outcomes were retrospectively analyzed, such as intrauterine pregnancy, ectopic pregnancy and first-trimester abortion. The relevance between the infertility etiology and the pregnant outcomes was investigated at the same time. **Results:** When serum  $\beta$ -hCG level was less than 45 IU/L, no intrauterine pregnancy occurred; at the range of 46–100 IU/L, the chemical pregnancy loss was found in 50.00% women, and the occurrence of intrauterine pregnancy, ectopic pregnancy and first-trimester abortion were 25.00%, 10.71% and 14.29%, separately. The incidence rate of the chemical pregnancy loss decreased with the raising of serum  $\beta$ -hCG level, and was below 4.17% when the serum  $\beta$ -hCG > 200 IU/L. Prevalence of ectopic pregnancy significantly declined when the serum  $\beta$ -hCG > 400 IU/L (0.19% vs 10.20%,  $P=0.007$ ). Prevalence of first-trimester abortion decreased when the serum  $\beta$ -hCG > 500 IU/L (4.66% vs 12.88%,  $P=0.002$ ). The serum  $\beta$ -hCG cut-off value of intrauterine pregnancy on day 14 after embryo transfer was 379.25 IU/L, and the ROC area was 0.868, 95%CI=0.828–0.907. Women with tubal infertility factor accounted for 71.43% in ectopic pregnancy. Male infertility factor was found in 74.00% first-trimester abortion women, and 68.75% in chemical pregnancy loss. **Conclusion:** Serum  $\beta$ -hCG on day 14 after transfer two day 3 fresh embryos, is an effective measure to predict pregnant outcomes. When serum  $\beta$ -hCG is under 45 IU/L, it is not necessary to continue luteal support; when the value between 46.00–379.25 IU/L, luteal support should be continued, however, ectopic pregnancy and early miscarriage should be alerted, especially in those women with tubal and male infertility factors.

**Key words:** *in vitro* fertilization and embryo transfer (IVF-ET); serum  $\beta$ -hCG level; chemical pregnancy loss; ectopic pregnancy; first-trimester abortion

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# 体外受精-胚胎移植中含单绒毛膜双胎的 三胎减胎术后妊娠结局分析

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**【摘要】**目的: 探讨体外受精-胚胎移植(IVF-ET)中移植2枚胚胎后发生三胎妊娠行单绒毛膜双胎中一胎减灭术后的妊娠结局。方法: 回顾性分析27例行单绒毛膜双胎中一胎减灭术的三胎妊娠患者的临床资料, 了解术后一般情况, 并对比术后仍保留双胎妊娠的与同期非减胎双胎妊娠的妊娠结局及新生儿情况。结果: 减胎术后有12例(44.44%)发生自然减灭仅留一胎; 保留双胎组与非减胎双胎组患者平均年龄、平均孕周及平均胎儿出生体质量无统计学差异( $P>0.05$ ); 早期均无流产发生, 晚期流产率分别为6.67%和10.00%, 抱婴回家率分别为93.33%、90.00%, 早产率分别为21.43%和37.04%, 低体质量儿出生比例分别为21.43%和33.33%, 围产儿死亡率分别为0.0%和3.7%, 组间均无统计学差异( $P>0.05$ ), 减胎后双胎组和非减胎双胎组均无畸形儿出生。结论: 行减灭单绒毛膜双胎中一胎的减胎术有近一半的患者另一胎会发生自然减灭, 余保留双胎的妊娠结局和新生儿一般情况与非减胎双胎妊娠组无统计学差异。

**关键词:** 多胎减胎术; 含单绒毛膜双胎的三胎妊娠; 妊娠结局

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## Outcomes of multifetal pregnancy reduction strategies in triplet pregnancies with monochorionic twins in IVF-ET cycles

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**【ABSTRACT】 Objective:** To investigate the outcomes of the triplet pregnancy after transplanting two embryos during *in vitro* fertilization-embryo transfer (IVF-ET) which underwent a row monozygotic twin fetal cytoreductive surgery. **Methods:** A retrospective analysis was performed in a row monozygotic twin fetal cytoreductive surgery in triplet pregnancy with a total of 27 cases. The remaining twin pregnancy patients after surgery were considered as group A, and 30 cases of non-reduced fetal twin pregnancy were collected as group B. The pregnant outcomes and newborn infants indexes were compared between the two groups. **Results:** Totally, fetal reduction in 12 cases (44.44%) occurred naturally after cytoreductive leaving only one child. The patients' average age, the mean gestational age and the mean birth weight had no significant differences between group A and group B; no early abortion happened in the two groups. Late abortion rate was 6.67% in group A and 10.00% in group B, respectively, with no significant difference. Hold infant home rate (93.33% vs 90.00%), preterm birth rate (21.43% vs 37.04%), the proportion of low birth weight (21.43% vs 33.33%), and perinatal child mortality rate (0.0% vs 3.7%) were not significantly different between the two groups. There were no newborn deformed in the two groups. **Conclusion:** Less than half of the triplet pregnancies underwent the monozygotic twin fetal cytoreductive surgery occurs naturally after cytoreductive leaving only one child, and the other are still available to retain the twins. The two groups had similar pregnancy outcomes and neonatal conditions.

**Key words:** multifetal pregnancy reduction; dichorionic triplet pregnancy; pregnancy outcome

# 经阴道穿刺取卵(OPU)术中卵泡冲洗次数与卵母细胞回收情况的分析

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**【摘要】**目的: 研究分析经阴道穿刺取卵(OPU)术中最佳卵泡冲洗次数。方法: 回顾性分析在本中心因男方精子质量较差单因素寻求辅助生殖治疗且符合相关纳入标准的368例患者的相关临床数据, 分析不同卵泡冲洗次数与卵母细胞回收数的相关性及其不同卵泡冲洗次数下回收卵母细胞的质量。结果: 共计回收1996个卵母细胞, 总回收率为 $(94.8 \pm 11.8)\%$ ; 其中卵泡冲洗0次、1次、2次、3次、4次、 $\geq 5$ 次对应的回收率分别为29.9% (598/1996)、31.1% (620/1996)、21.7% (433/1996)、9.0% (180/1996)、4.5% (90/1996)、3.8% (75/1996); 冲洗次数 $\leq 3$ 次和 $> 3$ 次, 成熟卵率分别为84.1% (1540/1831)和12.1% (20/165), 差异有统计学意义( $P < 0.05$ ); 卵子异常形态率分别为12.7% (233/1831)和21.2% (35/165), 差异有统计学意义( $P < 0.05$ )。结论: OPU术中卵泡冲洗可以提高卵母细胞的回收率, 但反复多次的冲洗不能增加卵母细胞回收数目, 同时可能增加卵母细胞形态异常的发生率, 最佳卵泡冲洗次数应 $\leq 3$ 次。

关键词: 卵泡冲洗; 冲洗次数; 卵母细胞回收

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## Analysis of the oocyte retrieval in OPU with different follicular flushing times

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**【ABSTRACT】 Objective:** To explore the best follicular flushing time in ovum pick-up (OPU) by analyzing the oocyte retrieval with different follicular flushing times. **Methods:** A total of 368 patients who underwent IVF/ICSI only due to oligo-, astheno-, and teratosperwia of the male sperm in our center were included in the study. Follicular flushing with different times was performed for retrieving oocytes. **Results:** A total of 1 996 oocytes were retrieved, and the total oocyte retrieval rate was  $(94.8 \pm 11.8)\%$ . The oocyte retrieval with different follicular flushing times from 0 to 5 and more were 29.9%, 31.1%, 21.7%, 9.0%, 4.5%, 3.8%, respectively. Statistical differences were found in the mature oocyte retrieval rate (84.1% and 12.1%, respectively), abnormal oocyte morphology retrieval rate (12.7% and 21.2%) between flushing  $\leq 3$  times group and  $>3$  times group ( $P < 0.05$ ). **Conclusion:** Follicular flushing is necessary for oocyte retrieval, but the follicular flushing times should not be more than three times.

**Key words:** follicular flushing; flushing times; oocyte retrieval



# 闽西南地区多囊卵巢综合征患者临床干预前、后血清抗苗勒管激素变化研究

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**【摘要】**目的: 研究闽西南地区多囊卵巢综合征(PCOS)人群临床干预前、后血清抗苗勒管激素(AMH)的变化。方法: 采用酶联免疫吸附法(ELISA)检测80例PCOS患者和60例正常对照患者的血清AMH水平, 测定PCOS患者的性激素及相关代谢指标, 予以3个月药物治疗后复查指标。结果: PCOS患者血清AMH水平显著高于正常对照组; PCOS患者血清AMH与睾酮(T)、黄体生成素(LH)呈正相关; PCOS患者服用3个月药物治疗后血清AMH有下降趋势, 但无统计学差异( $P > 0.05$ )。结论: PCOS患者血清AMH水平升高, 可能通过与雄激素的相关作用参与了PCOS的病理发生过程。3个月的降雄及胰岛素增敏治疗能在一定程度上降低PCOS患者的AMH水平。

**关键词:** 多囊卵巢综合征(PCOS); 抗苗勒管激素(AMH); 临床干预

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## Change of serum anti-Müllerian hormone before and after clinical intervention with polycystic ovary syndrome in southwest Fujian province

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**【ABSTRACT】 Objective:** To investigate the changes of serum anti-Müllerian hormone (AMH) after clinical intervention in women with polycystic ovary syndrome (PCOS) in southwest Fujian province. **Methods:** Totally 80 patients with PCOS and 60 healthy females (control group) were enrolled and serum AMH levels were measured by enzyme linked immuno sorbent assay (ELISA). Levels of reproductive hormones, and sugar metabolism indicators of patients were measured in PCOS patients. The changes of parameters after 3-month treatment in PCOS patients were analyzed. **Results:** The serum AMH level was significantly higher in PCOS group than in control group. Meanwhile, there was a significant difference and a positive correlation between serum AMH and T, LH level. There was no significant difference but downward trend in serum AMH level after 3-month treatment on PCOS patients. **Conclusion:** Serum AMH level was elevated in patients with PCOS. The relationship is found between serum AMH level and serum androgen indexes, suggesting AMH may be related to the role of androgen through participation in the pathogenesis of PCOS process. Three-month treatment can reduce AMH levels in PCOS patients to some extent.

**Key words:** polycystic ovary syndrome (PCOS); anti-Müllerian hormone (AMH); clinical intervention

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# 子宫动脉化疗栓塞术治疗剖宫产 瘢痕部位妊娠临床分析

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**【摘要】**目的: 探讨双侧子宫动脉化疗栓塞术(bilateral uterine artery chemoembolization, BUACE)后刮宫术治疗剖宫产瘢痕部位妊娠(cesarean scar pregnancy, CSP)的疗效评价。方法: 回顾性分析6年来采用BUACE后刮宫术治疗的CSP患者的临床资料。结果: 36例患者中, 33例(91.67%)没有发生并发症, 分析显示顶臀径(crown-rump length, CRL)在治疗成功组和失败组之间差异有统计学意义( $P < 0.05$ )。CRL < 20 mm和CRL  $\geq$  20 mm组之间成功率的比值比(odds ratio, OR)为14.33, CRL与CSP的BUACE+刮宫术治疗预后有相关性。结论: 对于CRL < 20 mm的CSP, BUACE后刮宫术是安全有效的治疗手段。

**关键词:** 剖宫产瘢痕部位妊娠(CSP); 子宫双侧子宫动脉化疗栓塞术(BUACE); 刮宫术

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## Treatment analysis of bilateral uterine artery chemoembolization followed curettage for cesarean scar pregnancy

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**【ABSTRACT】 Objective:** To evaluate the feasibility and safety of bilateral uterine artery chemoembolization (BUACE) followed curettage to treat cesarean scar pregnancy (CSP). **Methods:** This was a retrospective case study of 36 cases who were diagnosed as CSP and treated by BUACE followed curettage under transabdominal ultrasound guidance over past 6 years. **Results:** Totally 33 of the 36 cases (91.67%) were successfully treated. A statistically significant difference was found in crown-rump length (CRL) between the success group and the failure group by rank sum test. Univariate Logistic regression analysis demonstrated that CRL was strongly associated with the prognosis and the *OR* for no complications was 14.33, comparing  $CRL < 20$  mm versus  $CRL \geq 20$  mm ( $P=0.042$ ). **Conclusion:** BUACE followed curettage under transabdominal ultrasound guidance is safe and effective in the treatment of CSP with  $CRL < 20$  mm.

**Key words:** cesarean scar pregnancy (CSP); bilateral uterine artery chemoembolization (BUACE); curettage