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## 辅助生殖中心全面质量管理

### ——从质量认证体系到质量管理工具的应用

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· 临床研究 ·

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## 降调节联合激素替代内膜准备方案

### 在冻融胚胎移植中非子宫内膜异位

### 症人群的应用

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**【摘要】** 目的 比较降调节联合激素替代内膜准备方案和单纯激素替代内膜准备方案在非子宫内异位症人群中冻融胚胎复苏移植临床结局的影响。方法 回顾分析 2012 年 1 月至 2017 年 12 月期间在中国人民解放军陆军第七十三集团军医院生殖医学中心行冻融胚胎复苏移植的 3562 个周期, 其中促性腺激素释放激素激动剂 (GnRH-a) 降调节后激素替代内膜准备组 (试验组) 807 个周期, 单纯激素替代内膜准备组 (对照组) 2755 个周期。通过多因素 logistic 回归模型分析两种内膜准备方案与妊娠率和活产率的关系。结果 试验组女方年龄 [ ( 31.19±4.58 ) 岁 ]、子宫内膜厚度 [ ( 8.95±1.75 ) mm ] 均高于对照组 [ ( 29.84±4.23 ) 岁、( 8.46±1.41 ) mm,  $P$  均 < 0.001 ] ; 试验组优质胚胎移植率 ( 9.2% ) 低于对照组 ( 19.5%,  $P < 0.001$  ) ; 试验组妊娠率 ( 55.3% ) 高于对照组 ( 51.3%,  $P = 0.045$  ) ; 试验组的活产率相对于对照组有升高趋势, 但差异无统计学意义 (  $P = 0.78$  ) 。在多因素 logistic 回归模型中, 校正包括女方年龄、不孕年限、内膜转化日子宫内膜厚度、移植胚胎数、囊胚移植率、是否移植优质胚胎在内的混杂因素后, 试验组相对于对照组妊娠率的校正 OR 值为 1.209 ( 95% CI = 1.023~1.429 ) , 活产率的校正 OR 值为 1.246 ( 95% CI = 1.053~1.474 ) 。结论 降调节联合激素替代内膜准备方案冻融胚胎移植在非子宫内异位症人群中可以获得比单纯激素替代内膜准备方案更高的临床妊娠率和活产率。

**【关键词】** 冻融胚胎移植; 内膜准备; 降调节联合激素替代周期; 激素替代周期; 妊娠率; 活产率

### Clinical application of down-regulating hormone replacement cycle in frozen-thawed embryo transfer in patients without endometriosis

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**【 Abstract 】** **Objective** To compare the effects of pituitary down-regulation in combination with hormone replacement therapy endometrial preparation protocol and conventional hormone replacement therapy endometrial preparation protocol on outcomes of frozen-thawed embryo transfer (FET) in patients without endometriosis. **Methods** The retrospective study included 3562 FET cycles was carried out between January 2012 and December 2017 in Reproductive Medicine Center, 73rd Group Army Hospital of PLA. Among them, 807 cycles were under hormone replacement therapy in combination with pituitary down-regulation with gonadotropin-releasing hormone agonist (GnRH-a) (experimental group) and 2755 cycles were under conventional hormone

replacement therapy (control group). Association between endometrial preparation protocols and pregnancy and live birth was analyzed with multivariate logistic regression. **Results** In experimental group, the maternal age [(31.19±4.58) years old] and endometrial thickness [(8.95±1.75) mm] were higher than those in control group [(29.84±4.23) years old, (8.46±1.41) mm, all  $P<0.001$ ], the proportion of cycles with at least one good-quality embryo transferred (9.2%) was lower than that in control group (19.5%,  $P<0.001$ ). The pregnancy rate (55.3%) was higher than that in control group (51.3%,  $P=0.045$ ). The live birth rate showed an increasing trend in experimental group in comparison with that in control group, but the difference was not statistically significant ( $P=0.78$ ). In multivariate logistic regression with adjustment of confounding factors including maternal age, duration of infertility, endometrial thickness on the day of endometrial transition, number of embryos transferred, blastocyst transfer and at least one good-quality embryo transferred, the odds ratio (OR) for pregnancy comparing experimental group with control group was 1.209 (95% CI=1.023–1.429) and OR for live birth was 1.246 (95% CI=1.053–1.474). **Conclusion** Pituitary down-regulation in combination with hormone replacement therapy as endometrial preparation protocol for FET may achieve higher pregnancy rate and live birth rate than conventional hormone replacement protocol.

**【Key words】** Frozen-thawed embryo transfer; Down-regulating hormone replacement cycle; Hormone replacement therapy; Embryo pregnancy rate; Live birth rate

## · 临床研究 ·

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# 卵胞质内单精子显微注射受精失败 卵子早期补救性人工激活技术体系 的建立及其效率分析

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**【摘要】** 目的 建立一个能够对卵胞质内单精子显微注射( ICSI )卵子受精失败进行早期判断进而实施早期补救性人工激活的技术体系,并探讨其激活效率及应用价值。方法 首先回顾性分析 2017 年 1 月至 2018 年 3 月期间广西壮族自治区人民医院生殖医学与遗传中心 150 个采用时差胚胎动态监测系统( Time-lapse )系统培养的 ICSI 周期第二极体( Pb2 )释放时间分布及其与受精及胚胎发育结局的关系,探讨 Pb2 释放作为 ICSI 受精失败早期判断指标的可行性。其次采用随机对照试验,收集 2018 年 3 月至 2019 年 6 月期间 93 个 ICSI 助孕周期的 225 个受精失败卵子,根据人工激活方式不同分为未激活组( NAOA 组)、早期激活组( RAOA 组)、晚期激活组( LAOA 组),同时将其余 ICSI 注射卵子作为对照组,比较 4 组受精及胚胎发育参数,探讨 ICSI 受精失败早期补救性人工激活的效率和应用价值。结果 Time-lapse 监测 ICSI 注射后卵子有 Pb2 释放组的受精率( 99.91%)、双原核( 2PN )受精率( 97.76%)显著高于无 Pb2 释放组( 0.03%、0.00%)且差异均具有统计学意义(  $P$ 均 $<0.001$ ); ICSI 注射后卵子 Pb2 释放时间为(  $3.04 \pm 1.45$  ) h, Pb2 释放时间分布及占比分别为 0~3 h ( 58.58%)、3~5 h ( 36.29%)、5~8 h ( 3.92%)、>8 h ( 1.21%) ; NAOA 组 Pb2 释放率、受精率、2PN 受精率均为 0%, LAOA 组 2PN 受精率( 36.00%)、第 3 日( D3 )优质胚胎率( 8.00%)、第 5 日( D5 )囊胚转化率( 0%)、D5 优质囊胚转化率( 0%)均显著低于 RAOA 组( 60.00%、44.19%、51.16%、25.58%)且差异均有统计学意义(  $P=0.005$ ,  $P=0.002$ ,  $P<0.001$ ,  $P=0.005$ ),同时均显著低于对照组( 97.63%、48.62%、63.23%、37.94%)且差异均有统计学意义(  $P$ 均 $<0.001$ ); RAOA 组 Pb2 释放率( 84.00%)、受精率( 81.33%)、2PN 受精率( 60.00%)、2PN 卵裂率( 95.56%)均显著低于对照组( 100.00%、99.68%、97.63%、99.51%)且差异均具有统计学意义(  $P<0.001$ ,  $P<0.001$ ,  $P<0.001$ ,  $P=0.04$ ),但 D3 优质胚胎率、D5 囊胚转化率、D5 优质囊胚转化率 2 组间差异均无统计学意义(  $P$ 均 $>0.05$ )。结论 Time-lapse 监测 Pb2 释放可以作为早期判断 ICSI 卵子受精失败的指标,在 ICSI 注射后 5 h 对受精失败卵子进行早期补救性人工激活可以获得正常受精和理想的胚胎发育结局。

**【关键词】** 延时成像; 受精; 极体; 卵胞质内单精子注射; 人工激活

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**Establishment and efficiency analysis of rescue artificial activation system for intracytoplasmic sperm injection fertilization failed oocytes**

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**【Abstract】 Objective** To establish a technical system for early judgment and rescue artificial oocyte activation of oocytes after fertilization failed, intracytoplasmic sperm injection (ICSI) and to explore the activation efficiency and application value. **Methods** Firstly, a retrospective analysis was performed in 150 ICSI cycles cultured by Time-lapse system during January 2017 to March 2018 from Reproductive Medical and Genetic Center, the People's Hospital of Guangxi Zhuang Autonomous Region, the time distribution of the second polar body (Pb2) exclusion and its relationship with fertilization and embryo development outcomes, the feasibility of Pb2 exclusion as an early indicator of fertilization failure in ICSI was discussed. Secondly, 225 fertilization failed oocytes from 93 ICSI cycles during March 2018 to June 2019 were collected for randomized controlled trials according to different artificial activation modes. They were divided into three groups including non-activation group (NAOA group), rescue activation group (RAOA group) and late activation group (LAOA group). At the same time, the rest of injected oocytes from ICSI cycles include in this study were used as control group. Fertilization and embryo development parameters were used to explore the efficiency and application value of rescue artificial activation for after fertilization failure oocytes ICSI. **Results** Time-lapse monitoring showed that the fertilization rate and 2PN fertilization rate were 99.91% and 97.76% in the Pb2-exclusion group, 0.03% and 0% in the without Pb2-exclusion group after ICSI, with significant differences between the two groups ( $P<0.001$ ); the exclusion time of Pb2 after ICSI was  $(3.04\pm 1.45)$  h, and the distribution and proportion of Pb2 exclusion time were 0–3 h (58.00%), 3–5 h (36.29%), 5–8 h (3.92%) and >8 h (1.21%). The Pb2 exclusion rate, the fertilization rate and the 2PN fertilization rate in NAOA group were all 0%, and the 2PN fertilization rate (36.00%), day 3 (D3) high-quality embryo rate (8.00%), day 5 (D5) blastocyst formation rate (0%) and D5 high-quality blastocyst formation rate (0%) in LAOA group were significantly lower than those in RAOA group (60.00%, 44.19%, 51.16%, 25.58%) ( $P=0.005$ ,  $P=0.002$ ,  $P<0.001$ ,  $P=0.005$ ), and also in control group (97.63%, 48.62%, 63.23%, 37.94%) ( $P<0.001$ ); the Pb2 exclusion rate (84.00%), the fertilization rate (81.33%), the 2PN fertilization rate (60.00%) and the 2PN cleavage rate (95.56%) in RAOA group were significantly lower than those in control group (100.00%, 99.68%, 97.63%, 99.51%) ( $P<0.001$ ,  $P<0.001$ ,  $P<0.001$ ,  $P=0.04$ ). However, there was no significant difference in D3 high-quality embryo rate, D5 blastocyst formation rate and D5 high-quality blastocyst formation rate between the two groups ( $P>0.05$ ). **Conclusion** Time-lapse monitoring of the Pb2 exclusion can be used as an early judgment indicator of fertilization failure in ICSI cycles. Rescue artificial activation of fertilization failed oocytes in 5 h after ICSI can achieve normal fertilization and acceptable embryo development outcomes.

**【 Key words 】** Time-lapse imaging; Fertilization; Polar bodies; Intracytoplasmic sperm injection; Artificial activation

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# 解冻周期序贯移植治疗反复种植失败的临床研究

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**【摘要】** 目的 探讨解冻周期序贯移植、单纯卵裂期及单纯囊胚期胚胎移植对反复种植失败 (recurrent implantation failure, RIF) 患者临床结局的影响及序贯移植胚胎数与多胎妊娠的关系。方法 回顾性分析 2015 年 1 月至 2019 年 1 月期间在天津市中心妇产科医院生殖医学中心行常规体外受精或卵胞质内单精子注射-胚胎移植 (IVF/ICSI-ET) 的 RIF 患者共 317 例, 分为四组: A 组为卵裂期+囊胚期共 2 枚胚胎移植组, 共 50 例; B 组为卵裂期+囊胚期共 3 枚胚胎移植组, 共 61 例; C 组为卵裂期胚胎移植组, 共 124 例; D 组为囊胚期胚胎移植组, 共 82 例。分析比较四组患者一般资料、促排卵结果和妊娠及分娩结局等。结果 患者年龄、不孕年限、体质指数 (BMI)、不孕原因、基础激素水平等组间差异均无统计学意义 ( $P>0.05$ )。A、B、D 组的胚胎种植率 (36.0%、31.1%、31.3%) 显著高于 C 组 (17.0%,  $P<0.001$ 、 $P<0.001$ 、 $P=0.026$ )。临床妊娠率、持续妊娠率及活产率在 A 组 (62.0%、56.0%、52.0%)、B 组 (60.7%、49.2%、47.5%) 间差异无统计学意义, 但两组均明显高于 C 组 (28.2%、20.2%、17.7%,  $P$ 均 $<0.001$ )、D 组 (40.2%、31.7%、30.5%, A 组与 D 组比,  $P=0.015$ 、 $P=0.006$ 、 $P=0.014$ ; B 组与 D 组比,  $P=0.016$ 、 $P=0.034$ 、 $P=0.037$ )。A 组早期流产率 (3.2%) 较 C 组 (25.7%,  $P=0.028$ ) 明显降



低。B组多胎妊娠率(32.4%)比A组(16.1%)、C组(14.3%)、D组(21.2%)较高,但差异无统计学意义( $P>0.05$ )。B组早产率(34.5%)比A组(25.9%)、C组(13.0%)、D组(8.0%)高,但仅B组和D组间早产率差异有统计学意义( $P=0.020$ )。结论 解冻周期序贯移植法能够显著提高RIF患者临床妊娠率、持续妊娠率及活产率,尤其是序贯移植2枚胚胎在显著提高临床妊娠率和活产率的同时不增加多胎妊娠率。序贯移植可成为RIF患者的一种有效的治疗手段。

【关键词】 生殖技术, 辅助; 胚胎移植; 解冻周期; 反复种植失败; 序贯移植

### Clinical research of sequential embryo transfer in frozen thawed cycles of patients with recurrent implantation failure

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【Abstract】 **Objectives** To investigate the effect of sequential embryo transfer, cleavage stage and blastocyst stage embryo transfer on the clinical outcome of patients with recurrent implantation failure (RIF) and the relationship between the number of sequential transferred embryos and multiple pregnancy. **Methods** A retrospective case-control study was conducted and the outcome of 317 patients with a history of RIF were analyzed, of which 50 women underwent sequential embryo transfer of two embryos on Day 3 and Day 5/6 (group A), 61 women underwent sequential embryo transfer of three embryos on Day 3 and Day 5/6 (group B), 124 women underwent Day 3 embryo transfer only (group C) and 82 women underwent Day 5/6 embryo transfer only (group D) at the Reproductive Medical Center of Tianjin Central Hospital of Gynecology and Obstetrics from January 2015 to January 2019. General information, controlled ovarian hyperstimulation outcome and pregnancy outcome were analyzed among four groups. **Results** There were no significant differences in age, duration of infertility, body mass index (BMI), and basal hormone levels between the groups. The embryo implantation rate of groups A, B and D (36.0%, 31.1% and 31.3%) was significantly higher than that of group C (17.0%,  $P<0.001$ ,  $P<0.001$ ,  $P=0.026$ ). The clinical pregnancy rate, the ongoing pregnancy rate and the live birth rate of group A (62.0%, 56.0%, 52.0%) and group B (60.7%, 49.2%, 47.5%) were significantly higher than those in group C (28.2%, 20.2%, 17.7%, all  $P<0.001$ ) and group D (40.2%, 31.7%, 30.5%; group A vs. group D,  $P=0.015$ ,  $P=0.006$ ,  $P=0.014$ ; group B vs. group D,  $P=0.016$ ,  $P=0.034$ ,  $P=0.037$ ). The early abortion rate of group A (3.2%) was significantly lower than that of group C (25.7%,  $P=0.028$ ). The multiple pregnancy rate in group B (32.4%) was higher than that in group A (16.1%), group C (14.3%), and group D (21.2%), but the difference was not statistically significant ( $P>0.05$ ). The preterm birth rate in group B (34.5%) was higher than that in group A (25.9%), group C (13.0%), and group D (8.0%). The difference in preterm birth

rate between group B and group D was statistically significant ( $P=0.020$ ).

**Conclusion** Sequential embryo transfer of frozen-thawed embryo transfer cycle can effectively improve the clinical pregnancy rate of patients with RIF. Especially, the sequential embryo transfer of 2 embryos can significantly improve the clinical pregnancy rate and the live birth rate without increasing the multiple pregnancy rate. Sequential transplantation can be used as an effective treatment for RIF patients.

**【Key words】** Reproductive technology, assisted; Embryo transfer; Frozen thawed cycle; Repeated implantation failure; Sequential embryo transfer

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# 年龄 $\leq 35$ 岁行卵巢刺激患者迟发重度卵巢过度刺激综合征的最佳预测因子探析

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**【摘要】** 目的 探讨迟发重度卵巢过度刺激综合征(OHSS)的最佳预测因子。方法 采用回顾性队列研究分析了广州医科大学附属第三医院2017年12月至2018年12月期间年龄 $\leq 35$ 岁患者1138个体外受精/卵胞质内单精子注射(IVF/ICSI)周期的临床数据。比较OHSS组和非OHSS组的妊娠周期。用单因素方差分析和 $\chi^2$ 检验计算两组间的差异。采用多变量logistic回归分析确定预测迟发性OHSS的因素。受试者工作特征(ROC)曲线用于评价OHSS的预测因子。结果 在 $\leq 35$ 岁患

者中,诊断为 OHSS 的妊娠周期中,基础窦卵泡数 (AFC) ( $P=0.009$ )、人绒毛膜促性腺激素 (hCG) 注射日雌二醇水平 ( $P<0.001$ )、获卵数 ( $P=0.001$ )、可用胚胎数 ( $P=0.016$ )、优质胚胎数 ( $P=0.007$ ) 及孕囊数 ( $P<0.001$ ) 均高于非 OHSS 妊娠周期。OHSS 组的外源性卵泡刺激素 (FSH) 启动量 ( $P=0.002$ )、总促性腺激素 (Gn) 使用量 ( $P<0.001$ ) 和卵巢刺激时间 ( $P=0.034$ ) 均低于非 OHSS 组。Logistic 回归分析的结果显示:孕囊数 ( $OR=2.408$ , 95%  $CI=1.529\sim 3.793$ ,  $P<0.001$ ) 是迟发重度 OHSS 的独立预测因子。线性预测因子 ( $lp$ ) = 孕囊数  $\times 0.879 - 3.946$ 。孕囊数  $\geq 2$  的病例相对于 1 个孕囊的病例发生 OHSS 的风险度  $RR$  值为 2.25。结论 在  $\leq 35$  岁患者中,孕囊数是迟发 OHSS 的独立预测因素。 $\geq 2$  个孕囊时,迟发性重度 OHSS 的发生率较高。

【关键词】 卵巢过度刺激综合征; 促性腺激素; 获卵数; 孕囊; 妊娠

### Identifying the best predictor of late-onset severe ovarian hyperstimulation syndrome in $\leq 35$ years old women undergoing ovarian stimulation

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**【Abstract】 Objective** To identify the best predictor of late-onset severe ovarian hyperstimulation syndrome (OHSS) in  $\leq 35$  years old women undergoing ovarian stimulation. **Methods** In this retrospective cohort study, the data from 1138 *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles at the Third Affiliated Hospital of Guangzhou Medical University from December 2017 to December 2018 were analyzed. The pregnancy cycles were compared between OHSS group and non-OHSS group. The differences were calculated for two groups using one-way ANOVA and chi-squared test. Factors were identified to predict late onset OHSS using multivariable logistic regression. Receiver operating characteristic (ROC) curves were used to evaluate the predictors of OHSS. **Results** Antral follicle count (AFC) ( $P=0.009$ ), estradiol levels on human chorionic gonadotropin (hCG) injection day ( $P<0.001$ ), the number of oocytes retrieved ( $P=0.001$ ), available embryos ( $P=0.016$ ) and high-quality embryos ( $P=0.007$ ) were higher, whereas, priming dosage of exogenous follicle-stimulating hormone (FSH) ( $P=0.002$ ), total dosage of gonadotropin (Gn) used ( $P<0.001$ ) and stimulation duration ( $P=0.034$ ) were lower in OHSS group than in non-OHSS group. The number of gestational sacs was higher in OHSS group than in non-OHSS group ( $P<0.001$ ). The number of gestational sacs ( $OR=2.408$ , 95%  $CI=1.529-3.793$ ,  $P<0.001$ ) was found to be independent predictive factors of late onset OHSS in  $\leq 35$  years old women. Linear predictor ( $lp$ ) = number of gestational sacs  $\times 0.879 - 3.946$ . The risk ratio of OHSS was 2.25 for patients with the number of gestational sacs  $\geq 2$  compared with those with 1 gestational sacs. **Conclusion** In  $\leq 35$  years old women, the number of gestational sacs was the independent predictive factor of late-onset severe OHSS, which was higher when  $\geq 2$  gestational sacs occurred.

**【Key words】** Ovarian hyperstimulation syndrome; Gonadotropin; Number of oocytes retrieved; Gestational sacs; Pregnancy

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# 重组胰岛素样生长因子-1 对 *MEX3C* 基因敲除小鼠卵巢胰岛素样生长因 子-1 受体相关表达和雌激素分泌的 影响

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**【摘要】** 目的 探讨重组胰岛素样生长因子-1 (IGF-1) 对 *MEX3C* 基因敲除小鼠卵巢发育的影响。方法 采用聚合酶链式反应 (PCR) 法鉴定 4 周龄 FVB (SPF 级) 小鼠基因型, 将鉴定出的雌性小鼠随机分为 4 组 (每组 6 只): 野生型组、*MEX3C*

基因敲除小鼠纯合子(简称纯合子组)、纯合子+IGF-1处理组、处理对照组。比较 IGF-1 注射前后各组小鼠体质量及卵巢湿重变化;酶联免疫吸附法(ELISA)测定血清中雌二醇水平;HE 染色观察卵巢形态结构;免疫组织化学法和 Western blotting 法测定 MEX3C、重组胰岛素样生长因子-1 受体(IGF-1R)和 p-AKT(Ser473)蛋白表达。结果 IGF-1 注射前,纯合子组体质量[(6.33±0.31)g]明显轻于野生型组[(15.20±0.35)g],差异有统计学意义( $P<0.001$ )。ELISA 结果显示,与处理对照组[(8.1248±0.8477)ng/L]相比,纯合子+IGF-1 处理组[(17.2453±0.0731)ng/L]血清雌二醇水平显著较高,差异有统计学意义( $P<0.001$ )。HE 染色结果显示,与处理对照组相比,纯合子+IGF-1 处理组中原始卵泡数(19.83±2.94)和初级卵泡数(15.50±2.69)增多,且闭锁卵泡数(7.17±1.42)减少,差异均有统计学意义( $P$ 均 $<0.05$ )。Western blotting 结果证实,与处理对照组相比,纯合子+IGF-1 处理组 IGF-1R 和 p-AKT 蛋白表达显著升高,差异有统计学意义( $P=0.0141$ ,  $P=0.0025$ )。结论 IGF-1 促进 MEX3C 基因敲除小鼠卵巢卵泡发育,其机制是通过上调 IGF-1R 和 p-AKT 水平,提高血清雌激素水平促进卵泡发育。

【关键词】 重组胰岛素样生长因子-1; MEX3C 基因; 雌激素; 重组胰岛素样生长因子-1 受体; 小鼠

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### Effect of recombinant insulin-like growth factor-1 (IGF-1) on ovarian IGF-1 receptor expression and estrogen secretion in MEX3C knockout mice

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【 Abstract 】 **Objective** To investigate the effect of recombinant insulin-like growth factor-1 (IGF-1) on ovarian development in MEX3C knockout mice. **Methods** Mice were randomly divided into four groups (6 mice in each group): wild type group, homozygous group, homozygous+IGF-1 treatment group, and treatment control group. The weight and wet ovarian weight of mice in each group was compared before and after IGF-1 injection. The serum levels of estradiol was determined using enzyme linked immunosorbent assay (ELISA). The morphological structure of the ovary was observed by HE staining. The expression of MEX3C, IGF-1 receptor (IGF-1R), and the phosphorylation of AKT (Ser473)(p-AKT) were detected by immunohistochemistry and Western blotting. **Results** Before IGF-1 injection, the body weight of homozygote group [(6.33±0.31)g] was significantly lighter than that in wild type group [(15.20±0.35)g,  $P<0.001$ ]. Serum estradiol level was significantly higher in homozygous+IGF-1 treatment group [(17.2453±0.0731)ng/L] than in treatment control group [(8.1248±0.8477

7) ng/L,  $P=0.001$ ]. HE staining results showed that compared with the treatment control group, the primitive follicles ( $19.83\pm 2.94$ ) and primary follicles ( $15.50\pm 2.69$ ) were increased in homozygous+IGF-1 treatment group, while the atretic follicles ( $7.17\pm 1.42$ ) were decreased (all  $P<0.05$ ). Western blotting results further confirmed that compared with the treatment control group, IGF-1R and p-AKT protein expressions were significantly decreased in homozygous+IGF-1 treatment group ( $P=0.014$ ,  $P=0.002$ ). **Conclusion** IGF-1 promotes ovarian follicular development in *MEX3C* knockout mice. The mechanism is related to the up-regulation of IGF-1R and P-AKT protein expression. IGF-1 can increase serum estrogen and promote follicular development.

**【 Key words 】** Recombinant insulin-like growth factor-1; *MEX3C* gene; Estrogen; Recombinant insulin-like growth factor-1 receptor; Mice

**Fund program:** National Natural Science Foundation of China (81560261); Ningxia Key Research and Development Program (2020BEG03042)

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# 滋阴补肾序贯法对多囊卵巢综合征大鼠卵巢 StAR 蛋白及 *SIRT1* 基因表达的影响

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**【摘要】** 目的 探讨滋阴补肾序贯法对多囊卵巢综合征(PCOS)大鼠卵巢类固醇激素合成急性调控蛋白(StAR)及SIRT1基因表达的影响。方法 用脱氢表雄酮(DHEA)创建PCOS大鼠模型。80只21日龄清洁级SD大鼠随机分为空白对照组(16只)和PCOS组(64只)。将造模成功大鼠随机分为生理盐水组、滋阴组、补肾组和序贯组,每组各16只。各组连续灌胃20d。用酶联免疫吸附法(ELISA)测定血清雌二醇、孕酮和睾酮浓度。分别用Western blotting和RT-PCR测定各组卵巢StAR、SIRT1蛋白及mRNA表达。结果 生理盐水组血清雌二醇水平较空白对照组显著升高( $P=0.004$ );滋阴组、补肾组和序贯组血清雌二醇水平较生理盐水组显著降低( $P=0.005$ 、 $P=0.003$ 、 $P=0.002$ )。生理盐水组血清睾酮水平显著高于空白对照组( $P=0.005$ );序贯组血清睾酮水平较生理盐水组、滋阴组和补肾组均显著降低( $P=0.002$ 、 $P=0.005$ 和 $P=0.005$ )。与空白对照组相比,生理盐水组StAR蛋白含量高( $P<0.001$ ),而SIRT1蛋白显著减少( $P<0.001$ );序贯组StAR蛋白及mRNA水平较生理盐水组、滋阴组和补肾组均显著降低( $P<0.001$ 、 $P=0.008$ 、 $P<0.001$ );序贯组SIRT1蛋白表达水平较生理盐水组和补肾组均显著升高( $P$ 均 $<0.001$ );与空白对照组相比,生理盐水组StAR mRNA显著升高( $P<0.001$ ),而SIRT1 mRNA显著减少( $P<0.001$ );滋阴组和序贯组SIRT1 mRNA水平较生理盐水组均有显著升高( $P$ 均 $<0.001$ ),而序贯组比补肾组显著升高( $P<0.001$ )。结论 滋阴补肾序贯法可能通过SIRT1基因高表达调控卵巢局部StAR等类固醇激素合成相关蛋白,而降低PCOS大鼠血清高雄激素血症。

**【关键词】** 多囊卵巢综合征; 滋阴补肾; 序贯法; 类固醇激素合成急性调控蛋白; 沉默信息调节因子1

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### Effect of Ziyin Bushen sequential method on acute regulatory proteins of steroid hormone and SIRT1 gene expression in ovaries of polycystic ovary syndrome rats

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**【Abstract】 Objective** To investigate the effect of Ziyin Bushen sequential method on the expression of steroids acute regulatory synthesis protein (StAR) and silent information regulator 1 (SIRT1) in ovaries of polycystic ovary syndrome (PCOS) rats. **Methods** PCOS rat model was established by dehydroepiandrosterone (DHEA). Eighty 21-day-old clean SD rats were randomly divided into control group (16 rats) and PCOS group (64 rats). PCOS group was further divided into normal saline (NS) group, Ziyin group, Bushen group and Xuguan group with 16 rats in each group. Each group was given intragastric administration for 20 d. Serum estradiol, progesterone and testosterone levels were measured by enzyme linked immunosorbent assay (ELISA) method. The expression levels of StAR, SIRT1 protein and mRNA were detected by Western blotting and RT-PCR. **Results** The serum estradiol level in normal saline group

was significantly higher than that in control group ( $P=0.004$ ). The levels of serum estradiol in Ziyin group, Bushen group and Xuguan group were significantly lower than that in NS group ( $P=0.005$ ,  $P=0.003$  and  $P=0.002$ , respectively). The serum testosterone level in normal saline group was significantly higher than that in control group ( $P=0.005$ ); the level of serum testosterone in Xuguan group was significantly lower than that in NS group, Ziyin group and Bushen group ( $P=0.002$ ,  $P=0.005$  and  $P=0.005$ , respectively). Compared with control group, the StAR protein content in the normal saline group was higher ( $P<0.001$ ), while *SIRT1* mRNA was significantly decreased ( $P<0.001$ ). Compared with NS group, Ziyin group and Bushen group, the expression of StAR protein and mRNA in Xuguan group was significantly lower ( $P<0.001$ ,  $P=0.008$  and  $P<0.001$ , respectively); the expression of SIRT1 protein in Xuguan group was significantly higher than that in NS group and Bushen group (all  $P<0.001$ ); compared with the control group, *StAR* mRNA in NS group was significantly increased ( $P<0.001$ ), while *SIRT1* mRNA was significantly decreased ( $P<0.001$ ); the expression of *SIRT1* gene in Ziyin group and Xuguan group was significantly higher than that in NS group (all  $P<0.001$ ), while that in Xuguan group was significantly higher than that in Bushen group ( $P<0.001$ ).

**Conclusion** Ziyin Bushen sequential method may decrease the serum androgen level of PCOS rats by upregulating *SIRT1* gene expression and StAR in ovary expression.

**【Key words】** Polycystic ovary syndrome; Ziyin Bushen; Sequential Method; Steroids acute regulatory synthesis protein; Silent information regulator 1

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## · 流行病学研究 ·

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# 不孕症女性唾液 $\alpha$ -淀粉酶和生育

## 压力的关系研究

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**【摘要】** 目的 探索不孕症女性的唾液 $\alpha$ -淀粉酶(salivary alpha-amylase, SAA)与其生育压力之间的关系。方法 采用方便抽样法抽取2017年4月至2017年12月期间在郑州大学第一附属医院生殖医学中心门诊的不孕症女性458例。采用应激的生物学标志物SAA和生育压力量表(Fertility Problem Inventory, FPI)对纳入的不孕症女性进行调查。通过淀粉酶酶联免疫吸附法(ELISA)试剂盒检测不孕症妇女的SAA浓度。结果 不孕症女性的生育压力总分为(155.86±24.54)分。不同组间的家庭月收入、不孕原因和不孕类型差异有统计学意义( $t=4.123$ ,  $P=0.041$ ;  $F=4.079$ ,  $P<0.001$ ;  $t=4.491$ ,  $P=0.035$ )。Pearson相关分析结果显示SAA与社会关注、关系关注、父母角色的需要、有孩子的生活方式、性关注和生育压力总分呈显著正相关( $r=0.360$ ,  $r=0.348$ ,  $r=0.222$ ,  $r=0.166$ ,  $r=0.203$ ,  $r=0.426$ ,  $P$ 均 $<0.001$ )。多元线性回归结果显示,生育压力、不孕原因和不孕类型是影响SAA的影响因素。结论 SAA与生育压力相关,结合SAA和FPI更客观地检测到不孕症女性的心理压力。

**【关键词】** 唾液 $\alpha$ -淀粉酶; 生育压力; 不孕症

## Relationship between saliva alpha-amylase and infertility stress among infertile women

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**【Abstract】 Objective** To explore the relationship between salivary alpha-amylase (SAA) and infertility stress in infertile women. **Methods** A convenient sampling method was used to investigate the infertile women in the Reproductive Medicine Center of the First Affiliated Hospital of Zhengzhou University from April 2017 to December 2017. A total of 458 infertile women were examined using the SAA and the Fertility Problem Inventory (FPI). SAA level was measured using an enzyme-linked immunosorbent assay (ELISA) kit. **Results** The total infertility stress score was 155.86±24.54. The family monthly income, the cause of infertility and the type of infertility were statistically different between different groups ( $t=4.123$ ,  $P=0.041$ ;  $F=4.079$ ,  $P<0.001$ ;  $t=4.491$ ,  $P=0.035$ ). Pearson correlation analysis showed that SAA and social concern, relationship concern, need for parenthood, rejection of child-free life, sexual concern and total infertility stress were significantly correlated ( $r=0.360$ ,  $r=0.348$ ,  $r=0.222$ ,  $r=0.166$ ,  $r=0.203$ ,  $r=0.426$ , all  $P<0.001$ ). Multiple linear regression results showed that infertility stress, infertility causes and types of infertility were factors influencing SAA. **Conclusion** SAA was associated with infertility stress. Combined with SAA and FPI may assess the psychological stress more objectively for infertile women.

**【Key words】** Salivary alpha-amylase; Infertility stress; Infertility

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# 腮腺炎性睾丸炎非梗阻性无精子症 患者行同周期显微睾丸取精结合卵 胞质内单精子显微注射技术的临床 结局分析

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**【摘要】** 目的 探讨由腮腺炎性睾丸炎所导致的非梗阻性无精子症 (NOA) 患者行同周期显微睾丸取精 (microTESE) 结合卵胞质内单精子显微注射 (ICSI) 治疗的临床结局。方法 回顾性分析 2013 年 12 月至 2019 年 10 月期间在西北妇女儿童医院生殖中心接受同周期 microTESE 结合 ICSI 治疗的有腮腺炎病史的 NOA 患者, 根据是否合并睾丸炎分为合并睾丸炎 NOA 患者 (合并睾丸炎组) 和未合并睾丸炎 NOA 患者 (未合并睾丸炎组) 2 组, 观察这 2 组在实施同周期 microTESE 结合 ICSI 治疗后的临床结果。结果 52 例既往有腮腺炎病史的 NOA 患者实施了 microTESE 手术, 其中检见精子 26 例, 总精子获得率 (SRR) 为 50.0%。合并睾丸炎组 SRR 为 94.4% (17/18), 未合并睾丸炎组 SRR 为 26.5% (9/34), 组间比较差异有统计学意义 ( $P < 0.001$ )。检见精子患者尝试 ICSI 治疗, 每取卵周期首次移植即临床妊娠

16例,临床妊娠率为61.5%(16/26)。合并睾丸炎组和未合并睾丸炎组的临床妊娠率和早期流产率差异均无统计学意义[58.5%(10/17)比66.7%(6/9), $P=0.696$ ; 20.0%(2/10)比16.7%(1/6), $P=0.868$ ]。实验室数据中,合并睾丸炎组和未合并睾丸炎组的双原核率差异有统计学意义(73.9%比57.0%, $P=0.006$ ),而优质胚胎率组间比较差异无统计学意义(44.2%比56.8%, $P=0.144$ )。结论 腮腺炎性睾丸炎NOA患者的SRR较高,通过同周期ICSI-microTESE治疗的临床效果良好。

【关键词】 非梗阻性无精子症; 腮腺炎性睾丸炎; 睾丸显微取精术; 卵胞质内单精子显微注射

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### Clinical outcomes analysis of nonobstructive azoospermia patients associated with mumps orchitis undergoing microdissection testicular sperm extraction and intracytoplasmic sperm injection

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**【 Abstract 】 Objective** To investigate the clinical outcome of microdissection testicular sperm extraction (microTESE) combined with intracytoplasmic sperm injection (ICSI) in patients with nonobstructive azoospermia (NOA) associated with mumps orchitis. **Methods** The clinical data of NOA patients who underwent microTESE from December 2013 to October 2019 in the ICSI treatment cycle in Assisted Reproduction Center, Northwest Women and Children's Hospital were collected. All the patients who had a history of mumps were divided into two groups according to whether they had mumps orchitis or not. The clinical outcomes after the implementation of microTESE combined with ICSI were compared. **Results** A total of 52 NOA patients were finally collected in the present study and 26 patients successfully processed sperm retrieval, the total sperm retrieval rate (SRR) was 50.0% (26/52). The SRR was 94.4% (17/18) in orchitis group, and 26.5% (9/34) in non-orchitis group. The difference between the two groups was statistically significant ( $P<0.001$ ). ICSI treatment was tried in sperm patients, and the clinical pregnancy rate was 61.5% (16/26). There was no statistically significant difference in the clinical pregnancy rate and the early pregnancy abortion rate between orchitis group and non-orchitis group [58.5% (10/17) vs. 66.7% (6/9),  $P=0.696$ ; 20.0% (2/10) vs. 16.7% (1/6),  $P=0.868$ ]. In the laboratory data, the difference of two pronucleus rate between orchitis group and non-orchitis group was statistically significant (73.9% vs. 57.0%,  $P=0.006$ ), while the difference of high-quality embryo rate between the two groups was not statistically significant (44.2% vs. 56.8%,  $P=0.144$ ). **Conclusion** The SRR of NOA patients with mumps orchitis was higher than that of non-orchitis. And good results can

be expected when microTESE and ICSI treatment are performed at the same period.

**【 Key words 】** Nonobstructive azoospermia; Mumps orchitis; Microdissection testicular sperm extraction; Intracytoplasmic sperm injection

**Fund program:** National Natural Science Foundation of China (81673224); Natural Science Foundation of Shanxi Province (2019JM-029)

· 现场调查 ·

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# 人工流产患者男性伴侣的生殖健康 知识来源与知识水平、避孕态度和 行为的横断面研究

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**【摘要】** 目的 了解人工流产患者男性伴侣生殖健康知识水平、避孕态度和行为及其生殖健康知识来源途径,分析它们之间相关性。方法 采用调查问卷的方法对 1085 例就诊于上海交通大学附属第一人民医院要求终止妊娠的女性男伴进行调查,收集男性生殖健康知识水平、知识来源、避孕态度和实施等相关资料,并对结果进行分析。结果 “网络”(78.52%)为各种社会学特征的男性获取生殖健康知识的最主要途径,其次为“朋友、同事”(36.76%)、“医务人员”(34.26%)、“电视视频”(33.80%)、“父母长辈”(15.83%)占比最低。人工流产患者男性伴侣获取生殖健康知识的途径越多,其避孕、流产等生殖健康知识水平越高( $P=0.001$ ),主动避孕态度越高(91.49%, $P<0.001$ )。结论 网络、医务人员、

媒体宣传等为男性获取生殖健康知识的主要来源途径,且获取途径越多,男性生殖健康知识水平越高,越可能主动采取有效的避孕措施,减少非意愿妊娠流产的发生。

【关键词】 避孕; 流产,人工; 男性; 生殖健康

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**A cross-sectional study on reproductive health knowledge sources and knowledge reserves, contraceptive attitudes and behaviors of abortion female's male partners**

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**【Abstract】 Objective** To investigate the reproductive health knowledge, contraceptive attitudes and behaviors of male partners of abortion women and their sources of reproductive health knowledge, and to analyze the correlation among them. **Methods** A questionnaire survey was conducted to survey 1085 female's male partners who requested abortion in Shanghai General Hospital, and information on male reproductive health knowledge, knowledge sources, contraceptive attitudes and implementation was collected, and the results were analyzed. **Results** "Network" (78.52%,  $P < 0.001$ ) was the most important way for males with various sociological characteristics to acquire reproductive health knowledge, followed by "friends, colleagues" (36.76%), "medical staff" (34.26%), "TV video" (33.80%), and "parental elders" (15.83%) which accounted for the lowest proportion. The more ways for abortion female's male partners to acquire reproductive health knowledge, the higher level of reproductive health knowledge such as contraception and abortion ( $P = 0.001$ ), the higher active contraceptive attitude (91.49%,  $P < 0.001$ ). **Conclusion** The main ways for male to obtain reproductive health knowledge include the internet, healthcare workers, media publicity, etc. and the more access, the more abundant the reproductive health knowledge of men, the more likely they are to take effective contraceptive measures and reduce the incidence of unintended pregnancy abortion.

**【Key words】** Contraception; Abortion, Induced; Male; Reproductive health

**Fund program:** Project of Shanghai Municipal Health Commission (201540355); Humanities Innovation Research and Cultivation Project of Shanghai Jiao Tong University (WK2014-16)

·综述·

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## 子宫内膜干细胞治疗宫腔粘连的研究进展

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**【摘要】** 宫腔粘连患者的子宫内膜基底层受损, 纤维组织增生, 生育能力受到严重影响。然而, 目前对中重度宫腔粘连患者的常规手术治疗术后复发率高且效果差。随着再生医学的发展, 外源性移植干细胞治疗宫腔粘连取得了一定的进展, 干细胞修复子宫内膜将成为一种新的宫腔粘连的治疗方式。研究表明, 子宫内膜中存在干细胞, 并且其参与内膜的修复与再生。本文主要对子宫内膜干细胞的研究现状和治疗宫腔粘连的进展进行综述。

**【关键词】** 子宫内膜干细胞; 宫腔粘连; 子宫内膜再生; 干细胞治疗

### Research progress of endometrial stem cells based therapy in intrauterine adhesions

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**【 Abstract 】** Intrauterine adhesions is a gynecological disorder characterized by endometrial basal layer damage and fibrous proliferation, which results in infertility. However, the conventional surgery therapy of intrauterine adhesions has high recurrence rates and poor effects. With the development of regenerative medicine, exogenous transplanted stem cell therapy has obtained certain curative effect on intrauterine adhesions as a new treatment. Numerous studies have demonstrated that the existence of endometrial stem cells can support the tissue maintenance/regrowth. In this review, we provide an overview of the accumulating evidence for the endometrial stem cells. We also discuss the development of endometrial stem cell therapy for intrauterine adhesions.

**【Key words】** Endometrial stem cells; Intrauterine adhesions; Endometrial regeneration; Stem cell therapy

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## 人类卵子生成、受精及早期胚胎发育异常的遗传学研究进展

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**【摘要】** 成功的繁殖需要配子成熟、受精和早期胚胎发育。卵母细胞成熟包括一系列形态学和分子学变化,经历生发泡期、减数分裂 I 中期和减数分裂 II 中期 ( $M_{II}$ )。而后精子与  $M_{II}$  期的卵母细胞融合,启动胚胎发育,逐步形成各种器官及组织。因此,卵母细胞发育过程中任何一个步骤失败都会导致不孕。然而,人类卵母细胞发育停滞的遗传学病因大多仍不甚清楚。母源 RNA 和蛋白质对维持正常的胚胎早期发育至关重要。皮质下母源复合体组分基因及其他特定基因的突变与临床上一些体外受精/卵胞质内单精子注射 (IVF/ICSI) 反复失败的女性不孕症相关。本文结合近期研究就人类卵子生成、受精及早期胚胎发育异常的遗传学基础进行简要综述。

**【关键词】** 卵母细胞; 卵母细胞发育停滞; 女性不孕; 遗传学

### Genetic research progress on abnormal human oocyte formation, fertilization and early embryo development

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**【 Abstract 】** Successful reproduction requires gamete maturation, fertilization, and early embryonic development. Oocyte maturation consists of a

series of morphological and molecular changes. Oocytes undergo germinal vesicle stage, metaphase I, and metaphase II. Following the fusion of a sperm with a metaphase II oocyte, the embryo starts to develop, and gradually forms various organs and tissues. Therefore, failure in any of the steps will lead to infertility. However, the genetic aetiology of human oocyte maturation arrest is still largely unknown. Maternal RNA and protein play a vital role in maintaining normal early embryonic development. The mutation of subcortical maternal complex component genes and other specific genes are related to female infertility characterized by repeated failure of *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI). This article reviews the recent studies of the genetic basis that cause abnormal human oocyte formation, fertilization and early embryo development.

**【Key words】** Oocyte; Oocyte maturation arrest; Female infertility; Genetics

· 综述 ·

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## 胚胎植入前基因检测技术在体外受精-胚胎移植中的应用现状

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**【摘要】** 胚胎植入前基因检测技术发展至今已在辅助生殖领域广泛应用，研究者们也不断地在优化该技术以期得到更好的应用。从最初只能检测有限数目的染色体检查逐渐优化为全基因组筛查技术，胚胎检测方法也由侵入性向非侵入性的方法开始转变，生殖中心对胚胎植入前基因检测技术的临床应用结局均有报道。通过对比不同中心的研究结果，我们了解到不同诊断技术、检测技术还有新近出现的改良技术各自的优势和局限性。本文结合胚胎植入前基因检测技术发展的过程，对目前临床应用胚胎移植前遗传学检测技术的研究进展进行综述。

**【关键词】** 胚胎植入前筛查；受精，体外；胚胎移植；全基因组筛查；优生



## Application of preimplantation genetic testing technique in *in vitro* fertilization and embryo transfer

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**【Abstract】** Preimplantation genetic testing technology has been widely used in the field of assisted reproduction, the researchers also constantly improve the technology in order to get better application. So from testing only a finite number of chromosome initially gradually optimized for whole genome screening technology. Embryonic detection method also began to change from invasive to noninvasive method. Many reproductive medicine centers had reported the clinical outcomes of preimplantation genetic testing technology. By comparing the results from different institution, we learn the advantages and limitations of different diagnosis technology, testing technology and the latest technology. Combined with the development process of preimplantation genetic testing technology, this article reviews the current research progress in clinical application of preimplantation genetic testing technology.

**【Key words】** Preimplantation genetic testing; Fertilization *in vitro*; Embryo transfer; Whole genome screening; Eugenics

·综述·

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## 长链非编码 RNA 在卵泡发育及其相关疾病中的研究进展

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**【摘要】** 长链非编码 RNA ( LncRNAs ) 的表达异常与许多卵泡发育异常疾病密切相关, 包括多囊卵巢综合征 ( PCOS ) 和原发性卵巢功能不全 ( POI ) 等。随着对 LncRNAs 的深入研究, 越来越多研究表明, LncRNAs 在卵泡发育异常疾病中差异表达并发挥功能。研究结果显示 LncRNAs 可能参与女性卵泡发育、卵丘扩张、卵子成熟和黄体形成等多个过程, 可能影响 PCOS 患者颗粒细胞的增殖、迁移和凋亡, 并且参与其调控内分泌和代谢过程。本文综述了 LncRNAs 参与卵泡发育及相关疾病的研究, 通过揭示疾病发生发展的机制, 发现新的生物标志物和治疗靶点, 为卵泡发育异常相关疾病的诊断和治疗提供新思路。

**【关键词】** 长链非编码 RNA ; 多囊卵巢综合征 ; 原发性卵巢功能不全 ; 卵泡发育

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### **Research progress of long non-coding RNAs in folliculogenesis and related diseases**

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**【Abstract】** Long non-coding RNAs (LncRNAs) are large and diverse class of non-protein-coding transcripts that are longer than 200 nucleotides. LncRNA plays an important role in folliculogenesis and related diseases including polycystic ovary syndrome (PCOS) and premature ovarian insufficiency (POI). LncRNAs regulate various follicular biological processes, including cumulus expansion, oocyte maturation, luteinization, etc. In women with PCOS, LncRNAs are also involved in cell proliferation, cell invasion, cell apoptosis, endocrine and metabolism actions. Here, we review the LncRNAs which may contribute to the occurrence of PCOS and POI. This article may provide potential biomarkers and therapeutic targets thus shed new light to understand the underlying mechanisms of folliculogenesis and related diseases.

**【 Key words 】** Long non-coding RNAs; Polycystic ovary syndrome; Premature ovarian insufficiency; Folliculogenesis

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