生理学报 *Acta Physiologica Sinica*, October 25, 2018, 70(5): 489–496
DOI: 10.13294/j.aps.2018.0067 http://www.actaps.com.cn

研究论文

促性腺激素释放激素激动剂控制性超促排卵对小鼠胚胎着床的作用及其机制

吴 琼，徐华洲，杨江霞，李志丽，傅 天，杜惠兰，宋翠淼*

河北中医学院，中西医结合生殖疾病协同创新中心，河北省中西医结合肝肾病证重点实验室，石家庄 050200

摘 要：本研究旨在探讨促性腺激素释放激素激动剂(gonadotropin-releasing hormone agonist, GnRHa)控制性超促排卵(controlled ovarian hyperstimulation, COH)对小鼠胚胎着床的作用及机制。40只9周龄昆明雌性小鼠随机分为两组：COH组腹腔注射GnRHa醋酸丙氨瑞林+人绝经期促性腺激素(human menopausal gonadotropin, HMG)+人绒毛膜促性腺激素(human chorionic gonadotrophin, hCG)，对照组腹腔注射等体积生理盐水。注射hCG日16:00雌雄小鼠合笼，妊娠第4天每组处死10只小鼠，用放射免疫法检测血清雌二醇(estradiol, E_2)、孕酮(progestrone, P)的水平；用HE染色观察卵巢、子宫内膜组织形态；用Western blot和免疫组织化学法检测子宫内膜白血病抑制因子(leukemia inhibitory factor, LIF)、磷酸化信号转导和转录活化因子3 (phosphorylated signal transducer and activator of transcription 3, p-STAT3)、肝素结合表皮生长因子样生长因子(heparin-binding epidermal growth factor-like growth factor, HB-EGF)和glycodelin A蛋白的变化。妊娠第8天每组处死10只小鼠，观察胚胎着床情况。结果显示，与对照组比较，COH组小鼠妊娠第4天(胚胎着床期)血清E_2水平降低($P < 0.05$)，P水平升高($P < 0.05$)；卵巢组织各级卵泡少见，可见多个黄体，子宫内膜变薄，腺体数量减少($P < 0.05$)；子宫内膜LIF、p-STAT3、HB-EGF和glycodelin A蛋白表达下降($P < 0.05$)；妊娠第8天胚泡发育缓慢，数量减少($P < 0.05$)。以上结果提示，GnRHa COH可影响小鼠胚胎着床，其机制可能与着床期内源性激素失衡，子宫内膜结构和LIF、p-STAT3、HB-EGF、glycodelin A蛋白表达改变，导致子宫内膜容受性降低、胚胎-子宫对话异常有关。

关键词：促性腺激素释放激素激动剂；控制性超促排卵；胚胎着床
中图分类号：Q492.6

Effect of GnRHa controlled ovarian hyperstimulation on mouse embryo implantation and its mechanism

WU Qiong, XU Hua-Zhou, YANG Jiang-Xia, LI Zhi-Li, FU Tian, DU Hui-Lan, SONG Cui-Miao*

Hebei University of Chinese Medicine, Collaborative Innovation Center of Integrated Chinese and Western Medicine on Reproductive Disease, Hebei Key Laboratory of Integrative Medicine on Liver-kidney Patterns, Shijiazhuang 050200, China

Abstract: The purpose of the present study was to investigate the effects and underlying mechanism of gonadotropin-releasing hormone agonist (GnRHa) controlled ovarian hyperstimulation (COH) on embryo implantation in mice. Forty female Kunming mice aged 9 weeks were randomly divided into two groups (control and COH groups). The COH group received intraperitoneal (i.p.) injections of aminocyclin acetate (GnRHa), human menopausal gonadotropin (HMG) and human chorionic gonadotropin (hCG), while the control group was given equal amount of physiological saline by i.p. injection. One male mouse and two female mice were put into the same cage at 16:00 on the hCG injection day, and on the fourth day of pregnancy, 10 mice from each group were killed. The levels of serum estradiol (E_2) and progesterone (P) were measured by radioimmunoassay; HE staining was used to observe the morphology

Received 2018-06-20 Accepted 2018-08-20

This work was supported by the National Natural Science Foundation of China (No. 81473719), the Science and Technology Research Project of Higher Education in Hebei Provincial Department of Education (No. ZD2015013) and the Scientific Research Project in Hebei Provincial Administration of Traditional Chinese Medicine (No. 700201601204005).

*Corresponding author. Tel +86-311-89926228; E-mail: songcuimiao@126.com

of ovarian and endometrial tissues. The protein expression levels of endometrial leukemia inhibitory factor (LIF), phosphorylated signal transducer and activator of transcription 3 (p-STAT3), heparin-binding epidermal growth factor-like growth factor (HB-EGF) and glycodelin A were detected by Western blot and immunohistochemistry. Ten mice from each group were sacrificed on the eighth day of pregnancy, and the status of the uterus and the average number of blastocysts were observed. The results showed that, compared with control group, the serum E_2 level in COH group was significantly decreased ($P < 0.05$), while the P level was increased significantly ($P < 0.05$); the ovarian follicles at different developmental stages were rare, corpus lutea (CL) were visible and multiple, the endometrium was thinned, and the number of endometrial glands was reduced ($P < 0.05$); the contents of LIF, p-STAT3, HB-EGF and glycodelin A in the endometrium were decreased significantly ($P < 0.05$) on the fourth day of pregnancy; mouse blastocysts developed slowly and were decreased in number on the eighth day of pregnancy ($P < 0.05$). The above results suggest that GnRHa COH can affect embryo implantation in mice. The mechanism may be related to the imbalance of gonadal hormone, the changes in the structure of the endometrium and the expressions of LIF, p-STAT3, HB-EGF and glycodelin A in the implantation stage, which may lead to the decrease of endometrial receptivity and the abnormal dialogue between the embryo and the uterus.

Key words: GnRHa; controlled ovarian hyperstimulation; embryo implantation

促性腺激素释放激素激动剂 (gonadotropin-releasing hormone agonist, GnRHa) 控制性超促排卵 (controlled ovarian hyperstimulation, COH) 方法可抑制黄体生成激素 (luteinizing hormone, LH) 峰提早出现，从而改善卵子质量，提高排卵率和优质胚胎率，开启了体外受精 - 胚胎移植 (*in vitro* fertilization-embryo transfer, IVF-ET) 的里程碑，但仍摆脱不了妊娠率低的难题，其中子宫内膜容受性及胚胎 - 子宫对话异常被认为是引发胚胎着床失败、导致妊娠率低的关键因素 [1]。研究显示，COH 通过改变子宫内膜各种生物活性因子如整合素 β (integrin β, ITGβ)、基质金属蛋白酶 (matrix metalloproteinase, MMP)、血管内皮生长因子 (vascular endothelial growth factor, VEGF) 的表达以及转录组学等，进而抑制子宫内膜容受性 [2–5]。近年来对 COH 后子宫内膜变化特征的研究成为提高 IVF-ET 妊娠率的突破口，本研究通过观察 GnRHa COH 对小鼠着床期子宫内膜组织形态和白血病抑制因子 (leukemia inhibitory factor, LIF)、磷酸化信号转导和转录活化因子 3 (phosphorylated signal transducer and activator of transcription3, p-STAT3)、肝素结合表皮生长因子样生长因子 (heparin-binding epidermal growth factor-like growth factor, HB-EGF) 和 glycodelin A 表达及小鼠胚胎着床的变化，探讨 GnRHa COH 影响胚胎着床的作用及可能机制，为临床 IVF-ET 过程中优化 COH 方案、提高妊娠率提供理论依据。

1 材料和方法

1.1 动物 清洁级 9 周龄、体重 25~30 g 昆明系雌性小鼠 40 只，连续 2 个动情周期正常。同品系成年、体重 30~35g 雄性小鼠 20 只，由河北省实验动物中心提供，许可证号：SCXK(冀)2013-1-003。

1.2 药物和试剂 醋酸丙氨瑞林 (GnRHa) 购自上海吉尔生化有限公司；人绝经期促性腺激素 (human menopausal gonadotropin, HMG)、人绒毛膜促性腺激素 (human chorionic gonadotrophin, hCG) 购自丽珠集团丽珠制药厂；雌二醇 (estradiol, E_2)、孕酮 (progesterone, P) 放射免疫试剂盒购自天津九鼎医学生物工程有限公司；抗 LIF、p-STAT3、HB-EGF、GAPDH 抗体购自 Santa Cruz 生物技术公司；兔抗小鼠 glycodelin A 多克隆抗体购自 EterLife 公司；山羊抗兔二抗购自 Abcam；HE 染液购自北京中杉金桥生物技术有限公司。

1.3 主要实验仪器 γ- 放射免疫计数器 (FJ-2021)，西安二六二厂；双色红外荧光成像系统 (Odyssey)，美国 LI-COR 公司；显微图像分析系统 (HMIAS-2000)，武汉千屏影像技术有限责任公司；电泳槽及电泳仪，北京六一实验仪器厂。

1.4 分组、给药与标本获取 本研究动物实验方案获河北中医学院伦理委员会批准 (No. 20160310)。动情周期正常的雌性小鼠按随机数字表分组法分为 COH 组和对照组，每组 20 只。COH 组小鼠从动情后期当天开始，每日 9:00 腹腔注射 GnRHa (40 μg/100 g 体重)，连续 9 天。至第 9 天同时注射 HMG (40 IU/100 g 体重)，48 h 后注射 HCG (100 IU/100 g 体重)[6, 7]。对照组以等体积生理盐水腹腔注射。注射 hCG 日 16:00，COH 组、对照组小鼠按照雌雄 2:1 比例合笼，次日见雌鼠阴栓即判为妊娠第 1 天。妊娠第 4 天 (胚胎着床期)，每组随机选取 10 只小鼠断头取血，剖腹取卵巢、子宫。将左侧卵巢和子宫

放入 4% 的多聚甲醛用于 HE 观察和免疫组织化学法检测；右侧子宫用生理盐水反复轻轻冲洗，刮取子宫内膜，−80 °C 冰箱保存用于 Western blot 检测。妊娠第 8 天将剩余小鼠颈椎脱臼处死，解剖观察胚胎着床情况。

1.5 放射免疫法检测妊娠第 4 天小鼠血清 E_2、P 水平 用液相平衡竞争放射免疫法 (回收率 95%~105%；非特异性结合率 ≤ 3%；特异性结合率 ≥ 30%)，定量 I^{125} 标记抗原与未标记样品抗原竞争限量抗体上的结合位点。样品抗原浓度和标记抗原抗体复合物呈负相关函数关系，并表现在剂量反应曲线上，以该曲线为依据对样品进行定量。

1.6 HE 染色观察妊娠第 4 天小鼠卵巢、子宫内膜组织形态 取卵巢、子宫组织，4% 多聚甲醛固定 24 h 后，常规脱水，石蜡包埋，4 μm 切片，脱蜡，水化，苏木精染色，盐酸酒精分化，伊红复染，酒精脱水，二甲苯透明，中性树胶封片。

1.7 Western blot 法检测妊娠第 4 天小鼠子宫内膜 LIF、p-STAT3、HB-EGF 蛋白的含量 取小鼠子宫内膜加入细胞核裂解液匀浆离心，测定上清蛋白，取含等量蛋白的上清样品加等量的 2 × 上样缓冲液混匀，100 °C 变性 5 min，冷却备用。配制 SDS- 聚丙烯酰胺凝胶，每孔加样品电泳，转至 PVDF 膜，3% 牛血清白蛋白室温封闭 2 h，分别加入抗 LIF、p-STAT3、HB-EGF、GAPDH 抗体 (1:500)，4 °C 孵育过夜，洗膜 3 次，加入 HRP 二抗 (1:20 000) 结合，室温反应 2 h，充分洗膜，Odyssey 仪器直接扫描成像，以 GAPDH 为内参，用 Quantity One 软件分析各种蛋白相对量 [8]。

1.8 免疫组织化学法检测妊娠第 4 天小鼠子宫内膜 glycodelin A 蛋白的表达 按照本研究组常规步骤操作 [8]，滴加抗 glycodelin A 抗体 (1:200)，二抗来自 ABC 染色试剂盒 (Vector)，DAB 显色，镜下棕黄色为 glycodelin A 蛋白阳性表达。应用 HMIAS-2000 高清晰度彩色病理图文分析系统分析 glycodelin A 蛋白平均光密度值 (蛋白表达量)。

1.9 统计学处理 数据用 mean ± SD 表示，用 SPSS 20.0 软件进行统计处理。数据服从正态分布

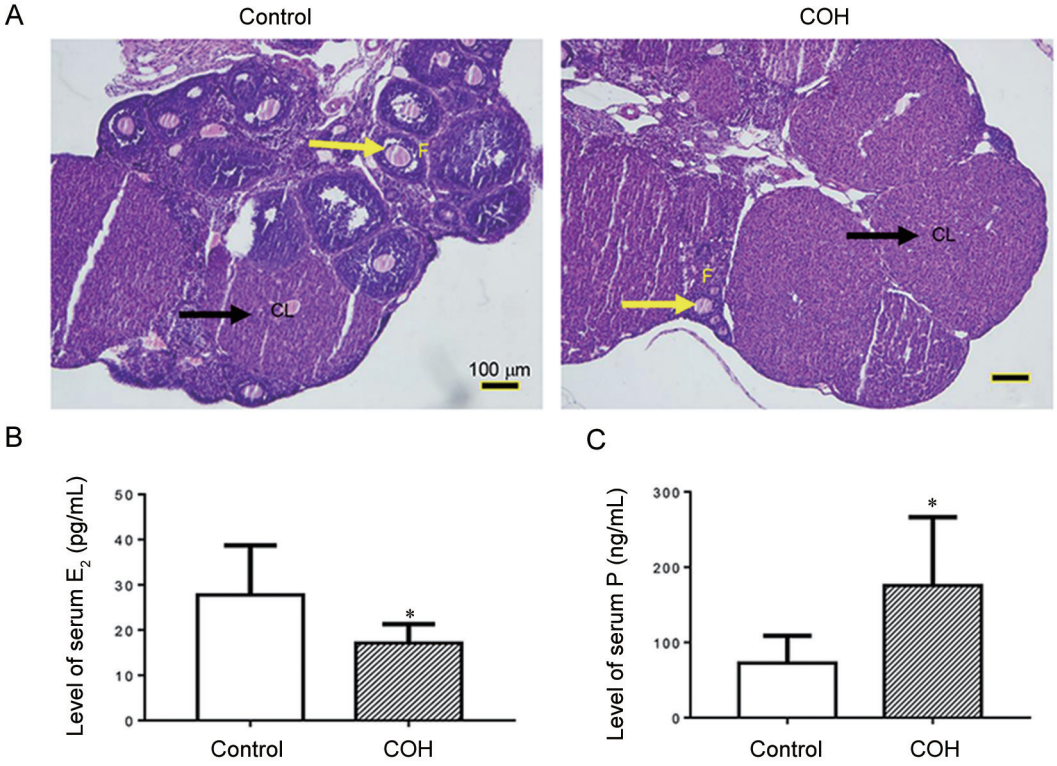

图 1. 小鼠妊娠第4天(胚胎着床期)卵巢组织形态学观察(HE染色)和血清雌二醇(E_2)、孕酮(P)水平

Fig. 1. The histomorphological observation of ovary (HE staining) and the serum levels of E_2 and progesterone (P) (radioimmunoassay) on the fourth day of gestation (embryo implantation) in mice. *A*: Microscopic images. The yellow arrow indicates the location of follicle (F), and the black one indicates the corpus luteum (CL). Scale bar, 100 μm. *B*: Statistical result of serum E_2 level. *C*: Statistical result of serum P level. Mean ± SD, $n = 10$. $^*P < 0.05$ *vs* Control group.

的采用两独立样本 *t* 检验进行组间差异的比较，不服从正态分布的采用秩和检验方法，计数资料采用 Fisher 确切概率法，$P < 0.05$ 时认为差异具有统计学意义。

2 结果

2.1 两组小鼠胚胎着床期卵巢组织形态和血清E_2、P水平的比较

对照组小鼠妊娠第 4 天卵巢可见各级卵泡 (黄色箭头指示)，数量较多，有黄体 (黑色箭头指示) 存在。COH 组小鼠妊娠第 4 天卵巢卵泡数量较少，可见多个黄体 (见图 1*A*)。与对照组比较，COH 组小鼠血清 E_2 水平降低 ($P < 0.05$，图 1*B*)，P 水平升高 ($P < 0.05$，图 1*C*)。

2.2 两组小鼠胚胎着床期子宫内膜组织形态、厚度及腺体数量的比较

对照组小鼠妊娠第 4 天子宫内膜增厚，腺体增粗、弯曲，基质疏松水肿，血管丰富，呈分泌期变化，COH 组小鼠妊娠第 4 天子宫腺体发育延迟，和基

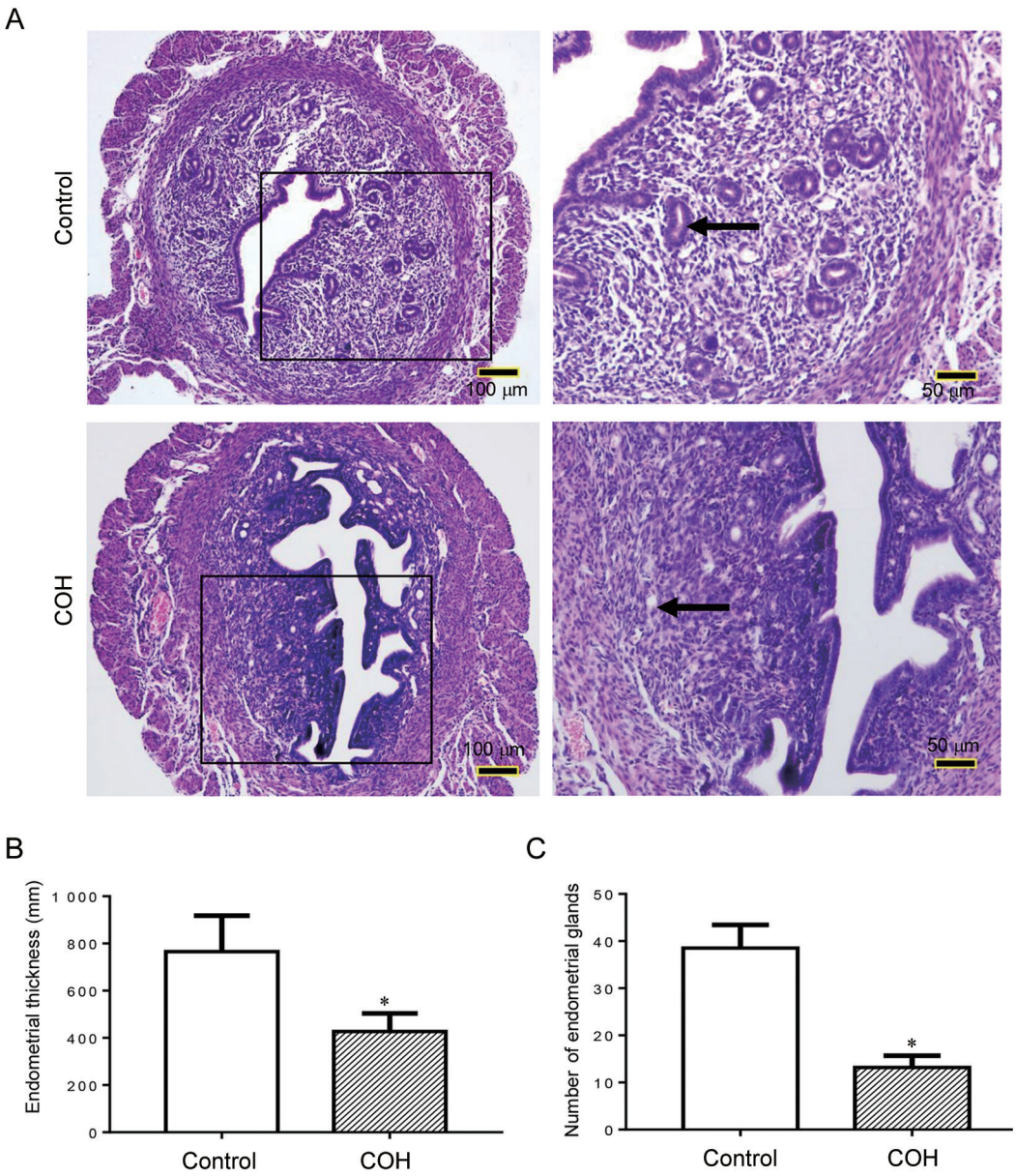

图　2. 小鼠妊娠第4天(胚胎着床期)子宫组织形态学观察(HE染色)，子宫内膜厚度及腺体数量

Fig. 2. The histomorphological observation of uterus (HE staining) and endometrial thickness and number of endometrial glands on the fourth day of gestation (embryo implantation) in mice. *A*: Microscopic images. The figures in the right column are enlargements of the framed areas. The arrows indicate the location of the endometrial gland. Scale bar, 100 or 50 μm. *B*: Statistical result of endometrial thickness. *C*: Statistical result of endometrial glands number. Mean ± SD, $n = 10$. $^*P < 0.05$ *vs* Control group.

质发育不同步 (图 2*A*)。与对照组比较，COH 组小鼠子宫内膜变薄 ($P < 0.05$，图 2*A*、*B*)，腺体数量减少 ($P < 0.05$，图 2*C*)。

2.3 两组小鼠胚胎着床期子宫内膜LIF、p-STAT3、HB-EGF蛋白表达的比较

Western blot 结果显示，与对照组比较，COH 组小鼠妊娠第 4 天子宫内膜 LIF、p-STAT3 和 HB-EGF 蛋白的表达均降低 ($P < 0.05$)(图 3)。

2.4 两组小鼠胚胎着床期子宫内膜glycodelin A蛋白表达的比较

免疫组织化学染色结果显示，glycodelin A 蛋白主要表达在小鼠子宫内膜腔上皮、腺上皮及基质，

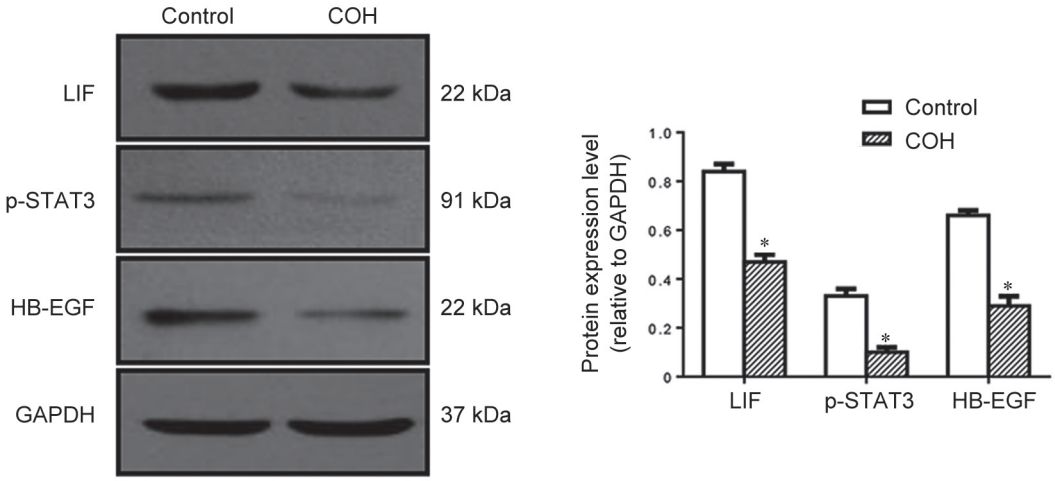

图 3. 小鼠妊娠第4天(胚胎着床期)子宫内膜LIF、p-STAT3 和HB-EGF蛋白表达水平

Fig. 3. Protein expression levels of LIF, p-STAT3 and HB-EGF in the mouse endometrium on the fourth day of gestation (embryo implantation) detected by Western blot. Mean ± SD, $n = 3$. $^{*}P < 0.05$ *vs* Control group.

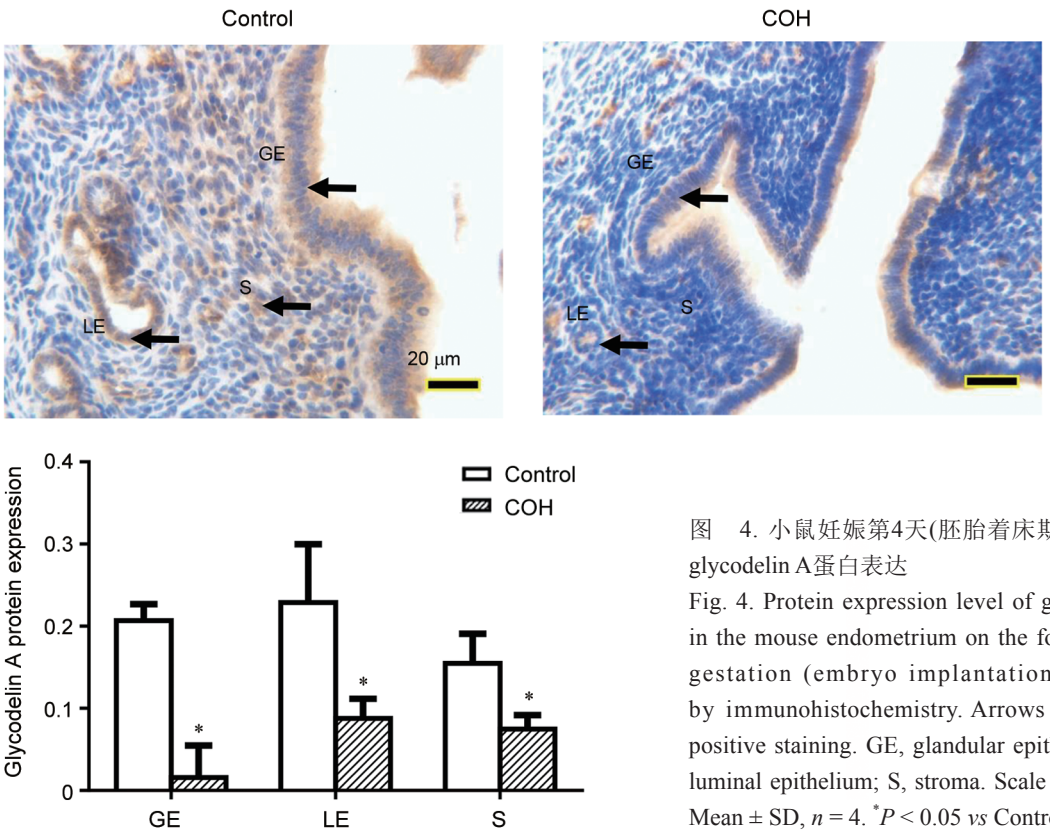

图 4. 小鼠妊娠第4天(胚胎着床期)子宫内膜glycodelin A蛋白表达

Fig. 4. Protein expression level of glycodelin A in the mouse endometrium on the fourth day of gestation (embryo implantation) detected by immunohistochemistry. Arrows showed the positive staining. GE, glandular epithelium; LE, luminal epithelium; S, stroma. Scale bar, 20 μm, Mean ± SD, $n = 4$. $^{*}P < 0.05$ *vs* Control group.

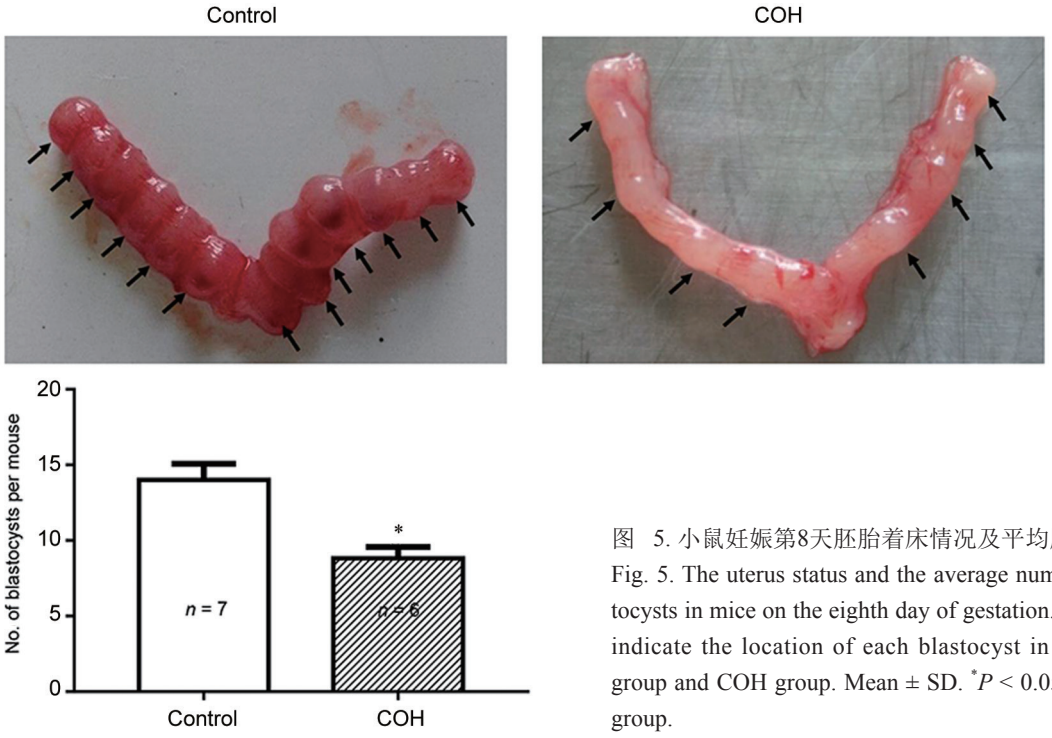

图 5. 小鼠妊娠第8天胚胎着床情况及平均胚胎着床数
Fig. 5. The uterus status and the average number of blastocysts in mice on the eighth day of gestation. The arrows indicate the location of each blastocyst in the control group and COH group. Mean ± SD. $^{*}P < 0.05$ *vs* Control group.

呈棕黄色。与对照组比较，COH 组小鼠妊娠第 4 天子宫内膜腔上皮、腺上皮及基质 glycodelin A 蛋白表达显著降低 ($P < 0.05$)(图 4)。

2.5 两组小鼠妊娠第8天胚胎着床情况观察

大体解剖结果显示，妊娠第 8 天，对照组小鼠胚泡发育良好呈串珠状，数量较多，在两侧子宫均匀分布，子宫充血明显。COH 组小鼠胚泡发育缓慢，数量较少 ($P < 0.05$)，分布不均，子宫苍白 (图 5)。

3 讨论

胚胎植入这一过程的正常发生对于妊娠成功至关重要。着床期子宫内膜在雌激素、孕激素作用下分泌多种细胞因子和黏附分子，呈现出容受性并通过胚胎 - 子宫对话接受胚胎植入，使胚胎成功着床。

3.1 GnRHa COH对小鼠胚胎着床期卵巢组织形态、血清E_2、P水平及子宫内膜组织形态的影响

本研究结果显示，COH 组小鼠胚胎着床期血清 E_2 水平降低，P 水平升高；卵巢组织各级卵泡少见，可见多个黄体，子宫内膜变薄，腺体数量减少。GnRHa 持续使用会大量消耗垂体 GnRH 受体，垂体脱敏而持续降调节，抑制卵泡刺激素 (follicle-stimulating hormone, FSH) 和 LH 分泌，致体内低雌激素状态，起到 GnRHa 暂时去势的作用 [9]，卵巢达到静息状态，卵泡早期同步募集。HMG 是由绝经期妇女尿中提取的物质，含有 FSH，使卵泡卵母细胞同步化发育，HCG 使成熟卵泡排出。本研究采用 GnRHa+HMG+HCG COH 后，由于大量卵泡同时发育成熟并排出，所以卵巢可见极少的卵泡和多个黄体，血清 E_2 (主要由卵泡颗粒细胞分泌) 水平降低，P (主要由黄体分泌) 水平升高。内源性激素的失衡对子宫内膜组织形态产生一定的影响，子宫内膜发育不良，厚度变薄，腺体数量减少，腺体缩小，与间质发育不同步，不利于胚胎着床。

3.2 GnRHa COH对小鼠子宫内膜容受性的影响

本研究结果显示，GnRHa COH 后，胚胎着床期小鼠血清 E_2 水平降低，血清 P 水平升高，LIF 蛋白在子宫内膜表达降低。子宫内膜同步分化到容受状态是成功着床的关键。作为雌激素反应基因，LIF 是一种参与子宫内膜容受性和胚胎植入的关键分子。研究表明在小鼠妊娠第 4 天 LIF 表达于子宫内膜腺上皮细胞，随着植入也表达于胚胎周围的子宫腔上皮细胞核中，与胚胎种植时间和部位是一致的，参与良好子宫内膜容受性的建立 [10]。作为 LIF 的直接下游靶标，STAT3 在建立子宫内膜容受性的过程中被磷酸化 [11]。当 STAT3 磷酸化被抑制或子宫条件性缺失 STAT3 时，会导致子宫内膜容受功能缺失，进而造成着床失败 [12, 13]。最近 Liang 等研究显示在过量 P 处理的小鼠中，子宫内膜 LIF 和

p-STAT3 的表达显著降低，可能导致胚胎着床的抑制 [14]，本研究结果与他们的这个研究结果是一致的。

3.3 GnRHa COH对小鼠着床过程中胚胎-子宫对话的影响

HB-EGF 已被确定为植入期间胚胎 - 子宫相互作用的早期介质和关键标志物 [15, 16]，是胚胎和子宫腺体之间通信的触发器 [17]。在植入期间，HB-EGF 在胚泡和子宫中均表达，子宫内膜 HB-EGF 利用自分泌、旁分泌和混杂信号模式与囊胚生长因子受体家族分子 (如 ErbB1) 之间相互作用影响囊胚黏附反应，调节胚胎 - 子宫功能分子对话 [18]。研究表明基因组 *Hbegf* 突变雌性小鼠缺乏 HB-EGF，延缓胚胎按时植入，导致妊娠结局受损 [19]。本研究结果显示，GnRHa COH 后，子宫内膜 HB-EGF 蛋白表达降低，对囊胚的黏附和胚胎 - 子宫功能分子对话产生不利的影响，进而使胚胎的植入受损。

Glycodelin A 参与胚胎 - 子宫界面极其复杂的免疫调节过程，在抑制母体对胚胎的免疫排斥反应中发挥重要作用。胚胎着床期 glycodelin A 在子宫内膜腺上皮表达逐渐增加，在月经前期达高峰。研究报道 glycodelin A 在不孕女性分泌期子宫内膜中的表达较低 [20]。用 rFSH 促排卵治疗的不育患者子宫腺上皮细胞 glycodelin A 表达显著低于排卵妇女 [21]。本研究结果与此观点一致，GnRHa COH 后，子宫内膜腔上皮、腺上皮及基质 glycodelin A 蛋白表达降低，使母体对胚胎的免疫抑制作用减弱，排斥反应增强，不利于胚胎着床。

综上所述，GnRHa COH 后，多个卵泡同时发育成熟排出，引起小鼠着床期雌、孕激素失衡，子宫内膜形态结构发生改变，不能为胚胎着床提供良好的微环境；并可能通过下调 LIF、p-STAT3、HB-EGF 和 glycodelin A 蛋白的表达影响子宫内膜容受性的建立和胚胎 - 子宫对话，进而对胚胎着床产生一定的不利影响，导致胚泡发育缓慢，着床数量减少。

参考文献

1 Pathare ADS, Zaveri K, Hinduja I. Downregulation of genes related to immune and inflammatory response in IVF implantation failure cases under controlled ovarian stimulation. Am J Reprod Immunol 2017; 78(1). doi: 10.1111/aji.12679.

2 Haas J, Casper RF. *In vitro* fertilization treatments with the use of clomiphene citrate or letrozole. Fertil Steril 2017; 108(4): 568–571.

3 Jakimiuk AJ, Nowicka MA, Zagozda M, Koziol K, Lewandowski P, Issat T. High levels of soluble vascular endothelial growth factor receptor 1/sFlt1 and low levels of vascular endothelial growth factor in follicular fluid on the day of oocyte retrieval correlate with ovarian hyperstimulation syndrome regardless of the stimulation protocol. J Physiol Pharmacol 2017; 68(3): 477–484.

4 Zanozin AS, Demura TA, Kolosovsky DY, Faizullina NM, Kogan EA. Impaired endometrial receptivity in primary infertility in women with undifferentiated connective tissue dysplasia and hereditary thrombophilia. Arkh Patol 2016; 78(6): 23–29 (in Russian with English abstract).

5 Papler TB, Bokal EV, Tacer KF, Juvan P, Virant Klun I, Devjak R. Differences in cumulus cells gene expression between modified natural and stimulated *in vitro* fertilization cycles. J Assist Reprod Genet 2014; 31(1): 79–88.

6 Fu ZY (付正英), Lu JR, Su XH. Effect of GnRH-a controlled ovarian hyperstimulation (COH) on homeoboxa10 (Hoxa-10) gene expression in mouse peri-implantation. Reprod Contracep (生殖与避孕) 2012; 32(7): 438–442, 463 (in Chinese with English abstract).

7 Du HL (杜惠兰), Song CM, Ma HR, Zhu F, Liu F, Hao XE. Experimental study on inducing ovulation in mice by Bushen Guchong series. J Hebei Trad Chin Med Pharmacol (河北中医药学报) 2001; 16(3): 8–9 (in Chinese with English abstract).

8 Duan YC (段彦苍), Song CM, He M, Du HL, Liu YH. Effects of Bushen Tiaojing Fang and Xiaoyao Wan on the expression of oocyte secreted factors in mouse ovary before ovulation. J Beijing Univ Trad Chin Med (北京中医药大学学报) 2017; 40(8): 635–640 (in Chinese with English abstract).

9 Zhang SD (张少娣), Geng JX, Lu J, Zhang HL, Zhang CL. Studies on GnRH agonist down-regulation protocols in regulation of endometrium receptivity. Chin J Reprod Contracep (中华生殖与避孕杂志) 2017; 37(8): 675–678 (in Chinese with English abstract).

10 Song H, Lim H, Das SK, Paria BC, Dey SK. Dysregulation of EGF family of growth factors and COX-2 in the uterus during the preattachment and attachment reactions of the blastocyst with the luminal epithelium correlates with implantation failure in LIF-deficient mice. Mol Endocrinol 2000; 14(8): 1147–1161.

11 Cheng JG, Chen JR, Hernandez L, Alvord WG, Stewart CL. Dual control of LIF expression and LIF receptor function regulate Stat3 activation at the onset of uterine receptivity and embryo implantation. Proc Natl Acad Sci U S A 2001; 98(15): 8680–8685.

12 Daikoku T, Cha J, Sun X, Tranguch S, Xie H, Fujita T, Hirota Y, Lydon J, DeMayo F, Maxson R, Dey SK. Conditional deletion of Msx homeobox genes in the uterus inhibits blastocyst implantation by altering uterine receptivity. Dev Cell 2011; 21(6): 1014–1025.

13 Sun X, Bartos A, Whitsett JA, Dey SK. Uterine deletion of Gp130 or Stat3 shows implantation failure with increased estrogenic responses. Mol Endocrinol 2013; 27(9): 1492–1501.

14 Liang YX, Liu L, Jin ZY, Liang XH, Fu YS, Gu XW, Yang ZM. The high concentration of progesterone is harmful for endometrial receptivity and decidualization. Sci Rep 2018; 8(1): 712.

15 Lim HJ, Dey SK. HB-EGF: a unique mediator of embryo-uterine interactions during implantation. Exp Cell Res 2009; 315(4): 619–626.

16 Griffith OW, Chavan AR, Protopapas S, Maziarz J, Romero R, Wagner GP. Embryo implantation evolved from an ancestral inflammatory attachment reaction. Proc Natl Acad Sci U S A 2017; 114(32): E6566–E6575.

17 Yuan J, Deng W, Cha J, Sun X, Borg JP, Dey SK. Tridimensional visualization reveals direct communication between the embryo and glands critical for implantation. Nat Commun 2018; 9(1): 603.

18 Hamatani T, Daikoku T, Wang H, Matsumoto H, Carter MG, Ko MS, Dey SK. Global gene expression analysis identifies molecular pathways distinguishing blastocyst dormancy and activation. Proc Natl Acad Sci U S A 2004; 101(28): 10326–10331.

19 Xie H, Wang H, Tranguch S, Iwamoto R, Mekada E, Demayo FJ, Lydon JP, Das SK, Dey SK. Maternal heparin-binding-EGF deficiency limits pregnancy success in mice. Proc Natl Acad Sci U S A 2007; 104(46): 18315–18320.

20 Leach RE, Jessmon P, Coutifaris C, Kruger M, Myers ER, Ali-Fehmi R, Carson SA, Legro RS, Schlaff WD, Carr BR, Steinkampf MP, Silva S, Leppert PC, Giudice L, Diamond MP, Armant DR. High throughput, cell type-specific analysis of key proteins in human endometrial biopsies of women from fertile and infertile couples. Hum Reprod 2012; 27(3): 814–828.

21 Valdez-Morales FJ, Gamboa-Domínguez A, Vital-Reyes VS, Cruz JC, Chimal-Monroy J, Franco-Murillo Y, Cerbón M. Changes in receptivity epithelial cell markers of endometrium after ovarian stimulation treatments: its role during implantation window. Reprod Health 2015; 12: 45.