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血 Hcy 水平对 PCOS 患者 FET 治疗流产风险的预测价值

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摘要 目的 探讨血同型半胱氨酸(Hcy)水平对多囊卵巢综合征(PCOS)患者行冻融胚胎移植(FET)治疗后流产的预测价值。方法 回顾性分析首次行FET治疗的536例PCOS患者和550例输卵管因素非PCOS患者(对照组)临床资料,比较两组间的基础资料和妊娠结局。对PCOS患者中流产组与活产组的基础资料进行比较,分析Hcy对PCOS患者FET治疗后流产的预测价值。结果 与对照组比较,PCOS组的体质指数(BMI)、睾酮(T)、Hcy、空腹胰岛素(FINS)、胰岛素抵抗的稳态模型(HOMA-IR)、总胆固醇(TC)、获卵数及流产率升高,而高密度脂蛋白胆固醇(HDL-C)、优质胚胎率及活产率降低,差异有统计学意义($P < 0.05$);与PCOS

患者中活产组比较,流产组患者Hcy、FINS及HOMA-IR升高,差异有统计学意义($P < 0.05$);Hcy、FINS、HOMA-IR均是PCOS患者流产的独立危险因素,且Hcy对PCOS患者流产的预测价值高于FINS和HOMA-IR,其ROC曲线下面积(AUC)为0.829(95% CI = 0.755 ~ 0.902, $P < 0.001$),最佳临界值为15.975 $\mu\text{mol/L}$,灵敏度为79.0%,特异度为87.4%,对PCOS患者流产具有较高的预测价值。结论 PCOS患者多伴有血Hcy水平明显升高,血Hcy水平对FET治疗后流产具有重要的预测价值。

关键词 多囊卵巢综合征;同型半胱氨酸;妊娠结局;流产

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多囊卵巢综合征(polycystic ovary syndrome, PCOS)是育龄期女性最常见的生殖内分泌疾病之一,主要表现为排卵障碍、雄激素水平升高及卵巢多囊样改变等,患病率为5%~15%,其中30%~40%的PCOS患者有过自然流产史,严重影响PCOS患者的身心健康^[1]。目前PCOS患者易发生自然流产的具体机制尚不明确。同型半胱氨酸(homocysteine, Hcy)是在蛋氨酸循环期间形成的含硫氨基酸,是心

in the YAP gene and its protein expression, and to preliminarily discuss the correlation between YAP and the development of non-cardia gastric cancer, so as to provide new ideas for the prevention and treatment of non-cardia gastric cancer. **Methods** 126 cases of non-cardia gastric cancer tissues and 104 cases of paracancerous normal tissues were collected and set as case group and control group, respectively. Immunohistochemical SP assay was used to detect the expression of YAP in non-cardia gastric cancer tissues and adjacent normal tissues. Taqman method was used for genotyping. The unconditional logistic regression method was used to calculate the odds ratio (OR) and its 95% confidence interval (CI) to detect the associations between YAP SNPs and the expression of YAP protein under Codominant, Dominant, Overdominant, Recessive, and Log-additive genetic models. **Results** The expression of YAP protein in non-cardia gastric cancer tissues was significantly higher than that in normal gastric tissues ($P < 0.001$). The expression level of YAP protein was negatively correlated with the degree of tumor differentiation ($P < 0.05$). There was no correlation between the expression level of YAP protein and gender, age, lymph node metastasis and tumor size respectively ($P > 0.05$). No associations were found between SNP rs11225163 and rs1820453 and the expression of YAP protein under Codominant, Dominant, Overdominant, Recessive and Log-additive genetic models. Haplotype was constructed by Haploview 4.2 software, and the association between haplotype and YAP protein expression was analyzed. **Conclusion** YAP may be involved in the development of non-cardia gastric cancer.

Key words Yes-associated protein; non-cardia gastric cancer; protein expression; single nucleotide polymorphism; association study

血管疾病的独立危险因素之一。国内外研究^[2-3]证实部分 PCOS 患者伴有血 Hcy 水平增高,且与不良妊娠结局有密切关系。该研究回顾性分析安徽医科大学第一附属医院妇产科生殖医学中心 2018 年 1 月—2020 年 12 月收治的首次行冻融胚胎移植(frozen-thawed embryo transfer, FET)治疗的 536 例 PCOS 患者和 550 例输卵管因素非 PCOS 患者临床资料,研究 PCOS 患者血 Hcy 水平对流产的预测价值。

1 材料与方法

1.1 病例资料 回顾性分析 2018 年 1 月—2020 年 12 月在安徽医科大学第一附属医院妇产科生殖医学中心首次行 FET 治疗的 536 例 PCOS 患者(PCOS 组)和 550 例输卵管因素非 PCOS 患者(对照组)临床资料。按妊娠结局,将最终流产的 PCOS 患者记为流产组(62 例),将最终活产的 PCOS 患者记为活产组(206 例)。PCOS 组纳入标准:25~35 岁,符合 2003 年鹿特丹会议 PCOS 诊断标准^[4],具备下述 3 条中的 2 条即可确诊为 PCOS,包含:①无排卵或稀发排卵;②具有高雄激素血症的临床症状和(或)生化依据;③卵巢多囊样改变。对照组纳入标准:25~35 岁,排卵正常,输卵管造影(hysterosalpingography, HSG)发现有输卵管梗阻或严重黏连,未诊断其他生殖方面疾病。两组排除标准:子宫内异症;卵巢储备功能下降;生殖道畸形;子宫内膜损伤;内分泌疾病如甲状腺功能异常、高泌乳素血症、库欣综合征等;夫妻一方或双方存在染色体异常;男方精液检查异常。所有患者均签署体外受精-胚胎移植(*in vitro* fertilization and embryos transfer, IVF-ET)知情同意书。

1.2 治疗方法 根据患者情况个体化制定相应的超促排卵方案^[5]。经阴道超声(transvaginal ultrasound, TVS)引导下穿刺取卵,获得的卵子进行常规体外受精、胚胎培养并观察胚胎发育情况。胚胎等级按加德纳评分标准, ≥ 3 BB 定义为优质胚胎^[5-6]。综合患者自身情况进行冷冻胚胎移植助孕,根据患者情况选择人工周期或自然周期进行内膜准备^[5]。在自然周期排卵后 5 d 或人工周期黄体酮转化后第 5 天进行胚胎解冻移植,TVS 引导下移植 1~2 枚优质胚胎,移植后 14 d 检测人绒毛膜促性腺激素(human chorionic gonadotropin, HCG)水平,若 HCG 阳性,则于移植后 30 d 行 TVS 检查确定是否临床妊娠。

1.3 观察指标 查阅安徽医科大学第一附属医院

生殖中心临床医学管理数据库,对患者进行病历资料统计。①一般情况:年龄、身高、体质量;②实验室检查:基础性激素[雌二醇(estradiol, E_2)、黄体生成素(luteinizing hormone, LH)、卵泡刺激素(follicle stimulating hormone, FSH)、睾酮(testosterone, T)]、Hcy、空腹血糖(fasting plasma glucose, FPG)、空腹胰岛素(fasting insulin, FINS)、空腹血脂[总胆固醇(total cholesterol, TC)、三酰甘油(triglyceride, TG)、高密度脂蛋白胆固醇(high density lipoprotein-cholesterol, HDL-C)、低密度脂蛋白胆固醇(low density lipoprotein-cholesterol, LDL-C)、脂蛋白(lipoprotein, LP)];③IVF-ET 结局:获卵数、优质胚胎数、产科结局(包括未着床、流产和分娩);④体质指数(body mass index, BMI) = 体质量/身高²(kg/m^2)、胰岛素抵抗(insulin resistance, IR)水平采用胰岛素抵抗的稳态模型(homeostasis model assessment of insulin resistance, HOMA-IR) = $(\text{FPG} \times \text{FINS})/22.5$ ^[7]表示;⑤优质胚胎率(%) = 优质胚胎总数/胚胎总数 $\times 100\%$ 、流产率(%) = 流产人数/(流产人数 + 活产人数) $\times 100\%$ 、活产率(%) = 活产人数/(流产人数 + 活产人数) $\times 100\%$ 。

1.4 统计学处理 采用 SPSS 22.0 统计学软件进行数据的整理与分析,非正态或者方差不齐计量资料采用 $M(P_{25}, P_{75})$ 表示,组间比较采用曼惠特尼 U 秩和检验,计数资料采用 $n(\%)$ 表示,组间比较采用 χ^2 检验,对两组间有统计学差异($P < 0.05$)的指标纳入多元回归分析独立影响因素。以 Hcy、FINS、HOMA-IR 值作为检验变量,流产作为状态变量,绘制 ROC 曲线,ROC 曲线下面积(AUC) > 0.5 表示对流产具有预测价值。

2 结果

2.1 PCOS 组与对照组基础资料及妊娠结局比较

两组患者在年龄、 E_2 、LH、FSH、FPG、TG、LDL-C 及 LP 方面差异无统计学意义($P > 0.05$)。PCOS 组患者 BMI、T、Hcy、FINS、HOMA-IR 及 TC 水平高于对照组, HDL-C 水平低于对照组,差异有统计学意义($P < 0.05$)。PCOS 组患者的获卵数大于对照组,而优质胚胎率小于对照组,差异有统计学意义($P < 0.05$)。PCOS 组患者的流产率大于对照组,而活产率小于对照组,差异有统计学意义($P < 0.05$)。见表 1。

2.2 PCOS 患者中流产组与活产组基础资料比较

PCOS 患者中,流产组与活产组在年龄、BMI、 E_2 、

表1 PCOS组与对照组基础资料及妊娠结局比较[$M(P_{25}, P_{75})$, % (n/N)]

项目	PCOS组($n=536$)	对照组($n=550$)	Z/χ^2 值	P 值
年龄(岁)	30(28,33)	30(28,32)	-0.436	0.663
BMI(kg/m^2)	23.38(21.64,25.22)	21.49(19.71,23.56)	-10.405	<0.001
E_2 (pmol/L)	164.94(109.19,220.10)	160.15(109.95,208.87)	-1.172	0.241
LH(IU/L)	9.15(6.94,11.84)	9.56(6.09,12.42)	-0.072	0.943
FSH(IU/L)	6.47(5.52,7.40)	6.46(4.93,7.73)	-0.466	0.642
T(nmol/L)	1.83(1.22,2.48)	1.37(1.01,1.77)	-9.192	<0.001
Hcy($\mu\text{mol}/\text{L}$)	12.38(9.44,15.93)	6.97(5.92,8.06)	-20.937	<0.001
FPG(mmol/L)	5.22(4.63,5.85)	5.23(4.58,5.88)	-0.296	0.767
FINS(mU/L)	13.35(11.16,15.46)	9.61(8.21,10.92)	-19.507	<0.001
HOMA-IR	2.99(2.74,3.27)	2.18(1.78,2.64)	-18.101	<0.001
TC(mmol/L)	4.84(4.36,5.41)	4.61(4.05,5.16)	-5.375	<0.001
TG(mmol/L)	1.51(1.31,1.70)	1.55(1.34,1.75)	-1.584	0.113
HDL-C(mmol/L)	1.24(1.03,1.44)	1.59(1.30,1.81)	-14.907	<0.001
LDL-C(mmol/L)	2.76(2.42,3.09)	2.74(2.37,3.07)	-0.765	0.444
LP(mg/L)	169.87(113.94,222.11)	178.25(120.91,236.20)	-1.781	0.075
获卵数(个)	22(19,24)	16(14,19)	-20.337	<0.001
优质胚胎率(%)	74.95(3 911/5 218)	82.00(3 179/3 877)	64.227	<0.001
流产率(%)	23.13(62/268)	14.73(47/319)	6.797	0.009
活产率(%)	76.87(206/268)	85.27(272/319)	6.797	0.009

表2 PCOS组不同妊娠结局基础资料及妊娠结局比较[$M(P_{25}, P_{75})$]

项目	流产组($n=62$)	活产组($n=206$)	Z 值	P 值
年龄(岁)	30(28,33)	30(28,33)	-0.170	0.865
BMI(kg/m^2)	22.52(21.07,24.49)	23.40(21.47,25.06)	-1.946	0.052
E_2 (pmol/L)	165.25(111.81,220.07)	173.57(108.28,211.21)	-0.083	0.934
LH(IU/L)	10.52(6.62,12.97)	9.38(7.00,12.08)	-0.693	0.488
FSH(IU/L)	6.36(5.61,7.58)	6.40(5.29,7.38)	-0.645	0.519
T(nmol/L)	1.91(1.25,2.48)	1.88(1.31,2.49)	-0.208	0.835
Hcy($\mu\text{mol}/\text{L}$)	18.57(16.49,23.00)	11.90(9.30,14.51)	-7.843	<0.001
FPG(mmol/L)	4.95(4.18,6.00)	5.25(4.54,5.82)	-0.734	0.463
FINS(mU/L)	16.65(14.07,17.95)	12.64(10.66,14.66)	-6.930	<0.001
HOMA-IR	3.10(2.93,4.06)	2.90(2.75,3.01)	-6.755	<0.001
TC(mmol/L)	4.96(4.51,5.43)	4.82(4.36,5.40)	-1.021	0.307
TG(mmol/L)	1.48(1.30,1.66)	1.49(1.26,1.71)	-0.289	0.773
HDL-C(mmol/L)	1.32(1.06,1.47)	1.23(1.04,1.43)	-0.593	0.553
LDL-C(mmol/L)	2.76(2.32,3.20)	2.73(2.44,3.04)	-0.467	0.640
LP(mg/L)	162.41(125.42,200.27)	169.16(113.66,223.08)	-0.727	0.467
获卵数(个)	21.5(19,24)	22(19,24)	-0.353	0.724

LH、FSH、T、TC、TG、HDL-C、LDL-C、LP、FPG及获卵数方面差异无统计学意义($P>0.05$),流产组Hcy、FINS及HOMA-IR高于活产组,差异有统计学意义($P<0.05$)。见表2。

2.3 PCOS患者流产的危险因素 PCOS患者流产的多元回归分析结果显示,Hcy、FINS、HOMA-IR均是PCOS患者流产的独立危险因素[($OR=1.428$, $95\%CI=1.268\sim1.609$, $P<0.05$)、($OR=1.362$, $95\%CI=1.144\sim1.620$, $P<0.05$)、($OR=3.359$, $95\%CI=1.170\sim9.647$, $P<0.05$)]。见表3。

2.4 Hcy、FINS、HOMA-IR对PCOS患者流产预

表3 PCOS患者流产的多元回归分析

参数	B 值	OR 值	95% CI	P 值
Hcy	0.356	1.428	1.268 ~ 1.609	<0.001
FINS	0.309	1.362	1.144 ~ 1.620	0.001
HOMA-IR	1.212	3.359	1.170 ~ 9.647	0.024

测价值 PCOS患者Hcy、FINS、HOMA-IR预测流产的AUC分别为0.829、0.790、0.782(见图1),均>0.5,最佳临界值分别为15.975 $\mu\text{mol}/\text{L}$ 、15.505 mU/L 以及3.077,其中Hcy的AUC高于FINS以及HOMA-IR,且具有较好的灵敏度和特异度。见表4。

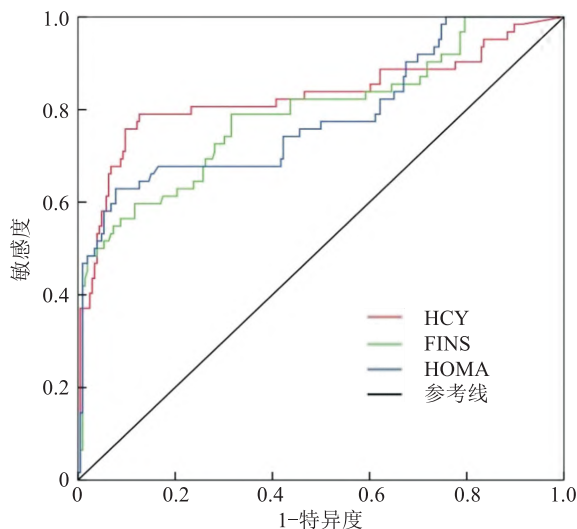


图1 Hcy、FINS、HOMA-IR对PCOS患者流产预测价值的比较

表4 Hcy、FINS、HOMA-IR对PCOS患者流产的预测价值

检验变量	AUC	95% CI	最佳临界值	灵敏度(%)	特异度(%)
Hcy	0.829	0.755 ~ 0.902	15.975 $\mu\text{mol/L}$	79.0	87.4
FINS	0.790	0.719 ~ 0.862	15.505 mU/L	59.7	88.3
HOMA-IR	0.782	0.709 ~ 0.857	3.077	62.9	92.2

3 讨论

PCOS是一种发病原因不明的生殖内分泌疾病,其常见临床症状包括代谢紊乱、肥胖以及不孕等,且PCOS患者妊娠期糖尿病、妊娠期高血压、早产、流产等不良妊娠结局的发生率高^[8]。PCOS患者在应用辅助生殖技术助孕的过程中,活产率低、流产率高,但具体原因尚未明确,肥胖、高雄激素血症(hyperandrogenemia, HA)、高胰岛素血症和IR等均公认是PCOS患者可能导致流产风险增加的危险因素。近年来,大量国内外研究^[8-12]表明PCOS患者流产可能与血Hcy水平升高相关。本研究中,与对照组比较,PCOS组的BMI、T、Hcy、FINS、HOMA-IR、TC、获卵数及流产率升高,而HDL-C、优质胚胎率及活产率降低,差异有统计学意义($P < 0.05$);与PCOS患者中活产组比较,流产组Hcy、FINS及HOMA-IR升高,差异有统计学意义($P < 0.05$);Hcy、FINS、HOMA-IR均是PCOS患者流产的独立危险因素,且Hcy对PCOS患者流产的预测价值最高。

PCOS患者体内HA、高胰岛素血症、IR等代谢紊乱会导致Hcy水平升高,同时Hcy水平的升高又会加重PCOS代谢紊乱状态,增加PCOS患者流产的风险。董丽霞等^[10]研究证实,PCOS患者血Hcy水平的升高与其异常的内分泌代谢关系密切,高胰

岛素血症或IR都可能引起血Hcy水平升高,且两者呈正相关。朱艳等^[11]研究表明,血Hcy水平升高是增加PCOS患者早期妊娠丢失风险的重要因素,且Hcy水平升高与患者的雄激素水平及IR有着密切关联。一项前瞻性研究^[12]表明,PCOS伴复发性流产患者Hcy、HOMA-IR水平显著升高,且血Hcy水平与HOMA-IR、BMI及游离睾酮水平呈显著正相关。

PCOS患者卵泡液和血液循环中高浓度Hcy可能是导致优质胚胎率及活产率降低、流产率升高的重要因素之一,并且可能增加出生缺陷的风险,影响助孕结局^[8-9,13]。卵泡液中高浓度的Hcy通过氧化应激产生大量的氧自由基及过氧化氢等物质会对卵母细胞产生毒性作用,进而降低卵母细胞质量及发育潜能,影响胚胎种植和正常妊娠的维持^[13];血液循环中高水平的Hcy可导致血管损害,影响母胎界面的血流和血管完整性,并可诱导血管内皮细胞产生并激活促凝血因子,引起胎盘血管微血栓形成增加,导致胎盘梗死,引发流产^[14]。本研究中,Hcy、FINS、HOMA-IR均是PCOS患者流产的独立危险因素,且Hcy对PCOS患者流产的预测价值大于FINS、HOMA-IR,与Chakraborty et al^[15]研究一致,表明PCOS患者流产是由多个病因所引起的共同最终结局,其中Hcy对流产具有重要的预测价值。临床上应监测高风险的PCOS妇女血Hcy水平,调整生活方式,积极治疗和调整代谢紊乱,个体化补充叶酸、维生素B₆、B₁₂和低分子肝素等,积极预防不良妊娠的发生。

本研究存在一定的局限性。纳入研究样本量相对较少,需多地区生殖中心间相互合作,扩大样本量,才能得出更准确的结论。此外,因系回顾性研究,对于患者血Hcy浓度升高治疗不明确,对结果产生一定的偏倚,且未能随访胎儿出生后的生长发育情况,缺乏对子代远期影响的研究,需要进一步设计良好的前瞻性研究来验证这些结果。

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Predictive value of serum HCY level on the risk of miscarriage in patients with PCOS undergoing FET

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Abstract Objective To investigate the predictive value of serum homocysteine (HCY) level on the risk of miscarriage in polycystic ovary syndrome (PCOS) patients undergoing frozen-thawed embryo transfer (FET). **Methods**

536 PCOS patients and 550 non-PCOS patients with fallopian tube factors (control group) retrospectively were analyzed. The basic characteristics and pregnancy outcomes were compared between the two groups. Also, the basic characteristics between the miscarriage group and the live birth group in PCOS patients were compared. The predictive value of Hcy on the miscarriage of PCOS patients after FET treatment was analyzed. **Results** Compared with the control group, body mass index (BMI), testosterone (T), Hcy, fasting insulin (FINS), homeostasis model assessment of insulin resistance (HOMA-IR), total cholesterol (TC), number of retrieved oocytes and miscarriage rate increased in PCOS group, while high density lipoprotein-cholesterol (HDL-C), high-quality embryo rate and live birth rate decreased, and the differences were statistically significant ($P < 0.05$). Among PCOS patients, Hcy, FINS and HOMA-IR increased in the miscarriage group compared with live birth group, and the differences were statistically significant ($P < 0.05$). Hcy, FINS and HOMA-IR were independent risk factors for miscarriage in PCOS patients, and the predictive value of Hcy for miscarriage in PCOS patients was greater than FINS and HOMA-IR, and the area under ROC curve (AUC) was 0.829 (95% CI = 0.755 - 0.902, $P < 0.001$), the cut-off value was 15.975 $\mu\text{mol/L}$ with a 79.0% sensitivity and a 87.4% specificity, which indicated high predictive value for miscarriage in patients with PCOS. **Conclusion** Serum Hcy level significantly increased in PCOS patients, which has an important predictive value for miscarriage after FET treatment.

Key words polycystic ovary syndrome; homocysteine; pregnancy outcomes; miscarriage