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在线全文

体外受精-胚胎移植患者中反复妊娠丢失的危险因素分析*

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【摘要】 目的 探讨体外受精-胚胎移植（*in vitro* fertilization-embryo transfer, IVF-ET）患者发生反复妊娠丢失（recurrent pregnancy loss, RPL）的危险因素。**方法** 本研究采用回顾性病例对照设计，纳入2012年1月–2021年3月在中山大学孙逸仙纪念医院进行IVF-ET的反复妊娠丢失患者作为病例组，因男性不育而行IVF-ET治疗的妇女为对照组。在最后一次流产后至少12周的首次月经周期前5 d采集患者空腹外周血，比较两组的临床特征及实验室相关指标，并采用单因素和多因素logistic回归模型分析可能影响RPL的潜在高危因素。采用线性趋势检验确定总睾酮（total testosterone, TT）水平与流产次数间的线性关系。**结果** 与对照组相比，RPL组的年龄、体重指数及腰臀比（waist-to-hip ratio, WHR）均增加，差异有统计学意义（P<0.05）。RPL组的TT水平较对照组降低（P=0.022），两组间基础卵泡刺激素、黄体生成素、雌二醇、孕酮、泌乳素水平及抗米勒管激素水平差异无统计学意义（P>0.05）。与对照组相比，RPL组的空腹胰岛素（fasting insulin, FINS）水平及胰岛素抵抗指数的稳态评估模型升高（P<0.001），两组间空腹血糖水平差异无统计学意义（P>0.05）。RPL组的中性粒细胞计数及中性粒细胞/淋巴细胞比值水平较对照组升高（P<0.01）。在调整其他因素后，年龄≥35岁〔比值比（odds ratio, OR）=1.91，95%置信区间（confidence interval, CI）：1.06～3.43〕、WHR>0.8（OR=2.30，95%CI：1.26～4.19）、FINS>10 mU/L（OR=4.50，95%CI：1.30～15.56）会增加RPL的风险（P<0.05）。TT水平的升高会降低RPL的发病风险（OR=0.59，95%CI：0.38～0.93，P=0.023）。线性趋势检验发现，TT与自然流产次数间存在线性相关关系（P_{trend}=0.003）。**结论** 在接受IVF-ET的患者中，高龄、TT水平的降低、WHR升高及FINS水平升高是RPL的危险因素。

【关键词】 反复妊娠丢失 睾酮 胰岛素抵抗 肥胖症

Analysis of Risk Factors for Recurrent Pregnancy Loss in Patients Undergoing *in vitro* Fertilization-Embryo Transfer

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【Abstract】 Objective Recurrent pregnancy loss (RPL) presents a formidable challenge for individuals undergoing *in vitro* fertilization-embryo transfer (IVF-ET), forming both a clinical dilemma and a focal point for scientific inquiry. This study endeavors to investigate the intricate interplay between clinical features, such as age, body mass index (BMI), and waist-to-hip ratio (WHR), and routine laboratory parameters, including sex hormones, blood composition, liver and thyroid functions, thyroid antibodies, and coagulation indicators, in RPL patients undergoing IVF-ET. By meticulously analyzing these variables, we aim to uncover the latent risk factors predisposing individuals to RPL. Identifying potential factors such as advanced maternal age, obesity, and insulin resistance will provide clinicians with vital insights and empirical evidence to strengthen preventive strategies aimed at reducing miscarriage recurrence. **Methods** This retrospective case-controlled study included RPL patients who underwent IVF-ET treatment at Sun Yat-sen Memorial Hospital, Sun Yat-sen University, between January 2012 and March 2021 as the case cohort, compared with women receiving assisted reproductive treatment due to male infertility as the control cohort. The fasting peripheral blood was collected 5 days before the first menstrual cycle at least 12 weeks after the last abortion. The clinical characteristics and relevant laboratory indexes of the two groups were compared. Employing both univariate and multivariate logistic regression analyses, we sought to unearth potential high-risk factors underlying RPL. Additionally, a linear trend analysis was conducted to assess the linear relationship between total testosterone (TT) levels and the number of miscarriages. **Results** In contrast to the control cohort, the RPL cohort exhibited significant increases in age, BMI, and WHR (P<0.05). Notably, TT levels were markedly lower in the RPL cohort (P=0.022), while no significant differences were

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observed between the two groups concerning basal follicle-stimulating hormone, luteinizing hormone, estradiol, progesterone, prolactin levels, and anti-Müllerian hormone levels (P>0.05). Moreover, fasting insulin (FINS) levels and HOMA-IR index were notably elevated in the RPL cohort relative to the control cohort (P<0.001), although no significant differences were observed in fasting blood glucose levels (P>0.05). Furthermore, the neutrophil (NEU) count and NEU-to-lymphocyte ratio were notably higher in the RPL cohort (P<0.01). Univariate logistic regression analysis identified several factors, including age≥35 years old, BMI≥25 kg/m^2, WHR>0.8, FINS>10 mU/L, HOMA-IR>2.14, NEU count>6.3×10^9 L^{-1}, and an elevated NEU/lymphocyte ratio (NLR), as significantly increasing the risk of RPL (P<0.05). Although TT levels were within the normal range for both cohorts, higher TT levels were associated with a diminished RPL risk (odds ratio [OR]=0.67, 95% confidence interval [CI]: 0.510-0.890, P=0.005). After adjustments for confounding factors, age≥35 years old (OR=1.91, 95% CI: 1.06-3.43), WHR>0.8 (OR=2.30, 95% CI: 1.26-4.19), and FINS>10 mU/L (OR=4.50, 95% CI: 1.30-15.56) emerged as potent risk factors for RPL (P<0.05). Conversely, higher TT levels were associated with a reduced RPL risk (OR=0.59, 95% CI: 0.38-0.93, P=0.023). Furthermore, the linear trend analysis unveiled a discernible linear association between TT levels and the number of miscarriages (P_{trend}=0.003), indicating a declining trend in TT levels with escalating miscarriage occurrences. **Conclusion** In patients undergoing IVF-ET, advanced maternal age, lower TT levels, increased WHR, and elevated FINS levels emerged as potent risk factors for RPL. These findings provide clinicians with valuable insights and facilitate the identification of patients who are at high risks and the formulation of preventive strategies to reduce the recurrence of miscarriages.

【Key words】 Recurrent pregnancy loss　Testosterone　Insulin resistance　Obesity

反复妊娠丢失(recurrent pregnancy loss, RPL)是指与同一性伴侣在妊娠28周前连续两次或两次以上的妊娠丢失,包括生化妊娠,其发生率约为2%～5%[1-2],近年来发病率在全球有上升趋势,是困扰临床工作的难题和研究热点。RPL常见原因可能有胚胎染色体异常、解剖异常、内分泌异常、感染因素、免疫、炎症、代谢和血栓因素以及环境因素的不良影响[3-5],但仍有40%～60%无法找到明确的致病因素。近年来,有许多研究对RPL的病因和影响因素进行了相关分析,且取得了重要进展,如血栓前状态会明显增加不良妊娠结局的发生风险,但对于性激素、免疫细胞、凝血指标等对RPL的综合影响仍然知之甚少,目前还没有公认的RPL的临床预测危险因素。在治疗方面,需针对具体病因进行治疗,如内分泌治疗、感染控制、抗血栓治疗、免疫治疗等。本研究旨在通过对接受体外受精-胚胎移植(*in vitro* fertilization-embryo transfer, IVF-ET)助孕的RPL患者的临床特征〔如年龄、体质量指数(body mass index, BMI)、腰臀比(waist hip rate, WHR)〕和常见实验室指标(如性激素、血常规、肝功能、甲状腺功能、甲状腺抗体、凝血指标)进行分析,明确RPL的潜在危险因素。通过对RPL危险因素如高龄、肥胖、胰岛素抵抗等进行预警,为预防自然流产的再次发生提供思路及依据。

1　资料与方法

1.1　研究对象

本回顾性病例对照研究于2012年1月–2021年3月在中山大学孙逸仙纪念医院进行。本项研究已通过中山大学孙逸仙纪念医院伦理委员会批准(伦理批号: SYSEC-KY-KS-2020-030)。RPL组的纳入标准是与同一性伴侣连续发生2次及以上在妊娠28周之前的胎儿丢失,包括连续发生的生化妊娠[6]。对照组为因男性不育而行辅助生殖技术治疗的妇女,无不良妊娠史,无反复着床失败(移植次数≥3次,移植4个及以上优质胚胎后未孕)[7]。所有对照均为无生殖疾病的健康女性。

RPL组排除以下情况: ①夫妻双方或既往流产的胚胎染色体异常; ②解剖异常: 包括子宫异常或宫颈功能不全。

1.2　数据收集

在最后一次流产后至少12周的首次月经周期前5 d空腹进行外周血样本的采集。每名女性的体质量、身高和腰围均在穿着轻便衣服和禁食状态下测量,计算BMI和WHR。

用细胞分析仪(Sysmex XN9000, Kobe, Japan)进行白细胞计数、中性粒细胞计数以及淋巴细胞计数。使用自动化学发光分析仪(Beckman DxI 800, Inc., USA)测定基础性激素及抗米勒管激素(anti-Müllerian hormone, AMH),用葡萄糖氧化酶法(AU5821; Beckman Coulter, Miami, FL, USA)测定空腹血糖(fasting blood glucose, FPG)。用化学发光免疫检测系统(ADVIA Centaur XP; Siemens, 北京, 中国)检测空腹胰岛素(fasting insulin, FINS)、促甲状腺激素(thyroid stimulating hormone, TSH)、抗甲状腺过氧化物(antithyroid peroxidase, Anti-

TPO)和抗甲状腺球蛋白(anti-thyroglobulin, Anti-TG)。凝血参数包括D-二聚体和纤维蛋白原通过凝血分析仪(CS-5100; Sysmex, 日本神户)测量。所有实验室指标的批内和批间变异系数分别<5%和≤10%。

胰岛素抵抗指数的稳态评估模型(HOMA-IR)以FINS(U/mL)×FPG(mmol/L)/22.5计算。在中国女性人群中, HOMA-IR的界值仍然没有定论。女性HOMA-IR〔中位数(P_{25}, P_{75})〕在中国东南部的一项回顾性横断面研究中为1.31(0.93, 1.88)[8], 在另一项全国性横断面研究中为1.63(1.17, 2.29)[9], 而在中山大学孙逸仙纪念医院既往的研究中, 诊断胰岛素抵抗的HOMA-IR临界值是2.14[10]。综合以上3项研究的结果, 本研究将HOMA-IR的分界值设定为2.14。

1.3 统计学方法

采用SPSS(26.0)及R语言(版本号4.0.5)统计软件进行数据分析。因全部变量均不符合正态分布, 采用中位数(P_{25}, P_{75})表示, 两组间比较采用Wilcox检验, 多组间比较采用Kruskal-Wallis检验, $P_{双侧}<0.05$为差异有统计学意义。采用R语言的glm函数进行单因素二元logistic回归分析(即每个变量逐一放入模型, 自变量为分类变量时, 以最小值组作为参照组; 自变量为连续变量时, 直接将连续变量纳入二元logistic回归模型)。多因素logistic回归首先采用SPSS的线性回归做共线性分析, 经判断自变量间不存在共线性, 采用R语言的glm函数进行多因素二元logistic回归分析。趋势性检验采用SPSS进行线性回归检验, $P_{trend}<0.05$为差异有统计学意义。

2 结果

2.1 研究对象基本情况

本研究共纳入462例受试者, 如表1所示, 受试者年龄

表1 研究对象的临床特征及实验室特征

Table 1 Clinical and laboratory characteristics of the study subjects

Clinical and laboratory characteristic	RPL group (n=265)	Control group (n=197)	Statistic*	P
Age/yr.	34 (31, 37)	33 (31, 36)	−2.546	0.011
BMI/(kg/m^2)	22.0 (20.2, 24.1)	21.1 (20.0, 23.0)	−2.51	0.012
WHR	0.82 (0.78, 0.86)	0.80 (0.76, 0.84)	−3.38	0.001
TT/(nmol/L)	1.26 (0.78, 1.65)	1.37 (0.88, 1.76)	2.283	0.022
E2/(pg/mL)	39.00 (27.00, 54.00)	41.00 (27.75, 60.50)	1.039	0.299
P/(ng/mL)	0.70 (0.50, 0.85)	0.63 (0.45, 0.95)	−0.337	0.736
AMH/(ng/mL)	3.24 (1.68, 6.37)	3.89 (2.29, 7.31)	1.173	0.241
FSH/(mIU/mL)	7.50 (6.40, 9.30)	7.29 (6.19, 8.62)	−1.455	0.146
LH/(mIU/mL)	4.10 (3.10, 5.50)	4.16 (3.17, 5.48)	0.156	0.876
PRL/(ng/mL)	12.40 (9.50, 16.60)	12.25 (9.81, 17.83)	0.726	0.468
FPG/(mmol/L)	5.00 (4.70, 5.30)	5.00 (4.73, 5.20)	−0.119	0.906
FINS/(mIU/L)	11.16 (7.96, 14.68)	8.84 (5.88, 12.05)	−4.668	<0.001
HOMA-IR	2.52 (1.77, 3.30)	1.95 (1.30, 2.76)	−4.339	<0.001
TSH/(mU/L)	1.78 (1.21, 2.34)	2.15 (0.89, 2.51)	0.366	0.714
Anti-TPO/(IU/mL)	34.00 (28.00, 78.50)	47.50 (42.25, 59.00)	0.961	0.337
Anti-TG/(IU/mL)	24.0 (15.0, 50.0)	24.0 (19.5, 25.5)	−0.587	0.557
AST/(U/L)	17 (15, 22)	18 (16, 20)	0.321	0.748
ALT/(U/L)	14.0 (9.0, 20.5)	13.0 (10.0, 17.0)	−0.86	0.390
GGT/(U/L)	17.0 (13.0, 23.0)	15.5 (13.0, 19.0)	−1.305	0.192
WBC/×10^9 L^{-1}	6.60 (5.45, 8.55)	6.80 (6.10, 8.25)	0.303	0.762
NEU/×10^9 L^{-1}	4.26 (3.25, 5.84)	3.78 (2.83, 4.80)	−3.149	0.002
LYM/×10^9 L^{-1}	1.85 (1.60, 2.30)	1.96 (1.62, 2.36)	0.991	0.322
NLR	2.26 (1.58, 3.20)	1.77 (1.40, 2.33)	−4.002	<0.001
FIB/(g/L)	2.76 (2.37, 3.19)	2.77 (2.47, 3.36)	0.779	0.436
D-dimer/(mg/L)	0.25 (0.17, 0.42)	0.24 (0.17, 0.42)	0.476	0.634

RPL: recurrent pregnancy loss; BMI: body mass index; WHR: waist-to-hip ratio; TT: total testosterone; E2: estradiol; P: progesterone; AMH: anti-Müllerian hormone; FSH: follicle stimulating hormone; LH: luteinizing hodi; PRL: prolactin; FPG: fasting blood glucose; FINS: fasting insulin; HOMA-IR: homeostatic model of insulin resistance index; TSH: thyroid-stimulating hormone; Anti-TPO: antithyroid peroxidase; Anti-TG: anti-thyroglobulin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: glutamyl transferase; WBC: white blood cell count; NEU: neutrophil count; LYM: lymphocyte count; NLR: neutrophil/lymphocyte ratio; FIB: fibrinogen. * SPSS 26.0 was used for data analysis, and Wilcox test was used for comparison between the two groups. The data are presented as median (P_{25}, P_{75}).

22 ~ 40岁, 平均年龄33.7岁, 其中RPL组265例(57.4%), 对照组197例(42.6%)。RPL组的年龄、BMI及WHR均高于对照组, 差异均有统计学意义(P< 0.05)。两组间性激素水平比较, RPL组的总睾酮(total testosterone, TT)水平较对照组降低(P= 0.022), 两组间基础卵泡刺激素(follicle-stimulating hormone, FSH)、黄体生成素(luteinizing hormone, LH)、雌二醇(estradiol, E2)、孕酮(progesterone, P)、泌乳素(prolactin, PRL)水平及AMH水平差异无统计学意义。与对照组相比, RPL组的FINS水平及HOMA-IR升高(P< 0.001), 两组间FPG水平差异无统计学意义。RPL组的中性粒细胞计数及中性粒细胞/淋巴细胞比率(neutrophil/lymphocyte ratio, NLR)水平在两组之间差异有统计学意义(P< 0.01)。

2.2　反复妊娠丢失危险因素的单因素logistic回归分析

单因素logistic回归分析发现(表2), 年龄≥35岁、BMI≥25 kg/m^2、WHR> 0.8、FINS> 10 mU/L、HOMA-IR> 2.14、NEU> 6.3×10^9 L^{-1}、NLR升高会增加RPL的风险(P< 0.05)。尽管RPL组和对照组的TT水平均在正常范围内, 但TT水平的升高会降低RPL的发病风险〔比值比(odds ratio, OR)= 0.67, 95%置信区间(confidence interval, CI): 0.51 ~ 0.89, P= 0.005〕。

表 2　反复妊娠丢失的单因素logistic回归分析

Table 2　Univariate logistic regression analysis for recurrent pregnancy loss

Variate	*B*	SE	*z*	*P*	OR (95% CI)
Age					
<35 yr.					1 (Ref)
≥35 yr.	0.518	0.188	2.754	0.006	1.68 (1.16-2.43)
BMI					
<25 kg/m^2					1 (Ref)
≥25 kg/m^2	0.810	0.247	3.276	0.001	2.25 (1.38-3.65)
WHR					
≤0.8					1 (Ref)
>0.8	0.609	0.187	3.263	0.001	1.84 (1.28-2.65)
TT	−0.396	0.141	−2.804	0.005	0.67 (0.51-0.89)
FINS					
≤10 mU/L					1 (Ref)
>10 mU/L	0.942	0.208	4.541	<0.001	2.57 (1.71-3.85)
HOMA-IR					
≤2.14					1 (Ref)
>2.14	0.891	0.212	4.201	<0.001	2.44 (1.61-3.69)
NEU					
≤6.3×10^9 L^{-1}					1 (Ref)
>6.3×10^9 L^{-1}	1.170	0.364	3.216	0.001	3.22 (1.58-6.57)
NLR	0.429	0.107	4.006	<0.001	1.54 (1.24-1.89)

B: partial regression coefficient; *SE*: standard error; OR: odds ratio; CI: confidence interval. The other abbreviations are explained in the note to Table 1.

2.3　反复妊娠丢失危险因素的多因素logistic回归分析

多因素logistic回归分析发现(表3), 在调整其他因素后, 年龄≥35岁、WHR> 0.8、FINS> 10 mU/L会增加RPL的风险(P< 0.05)。TT水平的升高会降低RPL的发病风险(OR= 0.59, 95%CI: 0.38 ~ 0.93, P= 0.023)。

表 3　反复妊娠丢失的多因素logistic回归分析

Table 3　Multivariate logistic regression analysis for recurrent pregnancy loss

Variate	*B*	SE	*z*	*P*	OR (95% CI)
Age					
<35 yr.					1 (Ref)
≥35 yr.	0.647	0.298	2.168	0.030	1.91 (1.06-3.43)
BMI					
<25 kg/m^2					1 (Ref)
≥25 kg/m^2	0.314	0.401	0.782	0.434	1.37 (0.62-3.00)
WHR					
≤0.8					1 (Ref)
>0.8	0.832	0.307	2.713	0.007	2.30 (1.26-4.19)
TT	−0.521	0.229	−2.272	0.023	0.59 (0.38-0.93)
FINS					
≤10 mU/L					1 (Ref)
>10 mU/L	1.504	0.633	2.376	0.018	4.50 (1.30-15.56)
HOMA-IR					
≤2.14					1 (Ref)
>2.14	−0.545	0.628	−0.867	0.386	0.58 (0.17-1.99)
NLR	0.285	0.161	1.773	0.076	1.33 (0.97-1.82)
NEU					
≤6.3×10^9 L^{-1}					1 (Ref)
>6.3×10^9 L^{-1}	0.648	0.596	1.086	0.277	1.91 (0.59-6.15)

All abbreviations are explained in the note to Table 1 and Table 2.

2.4　TT水平与流产次数的关系

对流产次数进行分组, 未发生过自然流产的197例, 自然流产2次的183例, 自然流产3次的60例, 自然流产次数≥4次的22例, 上述4组TT水平(nmol/L)分别为〔中位数(四分位间距)〕: 1.37(0.88, 1.76), 1.30(0.78, 1.67), 1.25(0.80, 1.65), 1.17(0.67, 1.38), 4组间TT水平的比较差异无统计学意义(H= 6.783, P= 0.079)。接着采用线性趋势检验发现(图1), TT与自然流产次数间存在线性相关(P_{trend}= 0.003), 随着流产次数的增加, TT呈现出下降趋势。

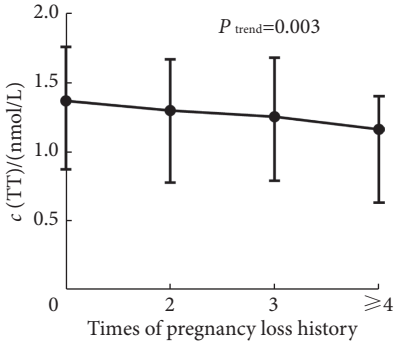

图 1 TT水平与自然流产次数的趋势关系

Fig 1 Trend relationship between total testosterone levels and the times of spontaneous abortion

The data are presented as median (P_{25}, P_{75}).

3 讨论

本研究结果显示,RPL组中性粒细胞计数及NLR较对照组明显升高。NLR的升高提示机体存在炎症反应且与炎症反应程度正相关[11],这提示RPL组患者体内存在长期炎症免疫反应过度激活的可能。此外,有研究在子痫患者多器官受累过程和血管炎性改变中也曾观测到中性粒细胞被过度激活[12],这些结果表明中性粒细胞激活可能参与了母胎界面血管重塑异常及免疫反应过度激活等过程,从而参与了病理妊娠的形成。但在排除其他混杂因素影响后,多因素logistic回归分析显示中性粒细胞计数并非RPL的危险因素。对于中性粒细胞在早期妊娠丢失中的作用目前尚无定论[13],有研究发现反复流产患者绒毛组织中的粒细胞刺激因子(granulocyte stimulating factor, G-CSF)水平降低,影响滋养层细胞的增殖和迁移能力[13]。G-CSF可通过募集蜕膜中Treg细胞,调控母体免疫应答,同时可与滋养层细胞表面的G-CSF受体结合,通过激活信号通路,调节滋养层细胞的迁移和侵袭,帮助胚胎着床和妊娠维持[14]。也有学者认为中性粒细胞的升高是RPL的一个危险因素[12],并对不良妊娠结局发挥重要作用,机制可能包括妊娠期的中性粒细胞激活后通过促进补体反应导致胎盘氧化损伤和促进炎性反应[15]。有关中性粒细胞在胚胎植入及早期妊娠维持中的作用机制有待进一步研究。

RPL组的FINS水平也显著升高,提示存在胰岛素抵抗(insulin resistance, IR),女性长期的高胰岛素血症,不仅影响机体正常内分泌功能,还与机体的能量代谢、高凝状态、炎性反应相关[16]。对于妊娠期女性而言,高胰岛素血症可影响子宫内膜蜕膜化进程和对胚胎的接受能力,子宫内膜的雌激素受体、孕激素受体异常下降,雄激素受体异常升高,胰岛素样生长因子结合蛋白-1下降,并引起血管内皮功能障碍,这些都可以对胚胎着床和胎儿发育产生不良影响[17-18]。目前已有研究证实IR能够引起子宫内膜蜕膜化进程障碍,还可以通过PI3K途径引起卵母细胞的葡萄糖转运和摄取异常,直接影响卵母细胞的发育和成熟[19]。这也解释了本研究所发现的FINS升高是RPL的危险因素之一。

此外,本研究提示WHR升高也是RPL的危险因素之一。WHR的升高常伴随着IR的发生,孟德尔分析表明腹部肥胖与IR也存在明显的因果关联[20],这也能很好的从病理生理机制层面解释WHR的升高与IR同时成为RPL的危险因素。在本研究中,经过共线性检验,FINS水平与WHR不存在共线性,经过多因素logistic回归矫正混杂因素影响后,WHR和FINS水平独立影响RPL的发生。WHR升高的女性体内脂肪堆积会引起线粒体等细胞器损伤,从而出现大量的活性氧,活性氧与脂肪酸可共同参与引发内质网的一系列应激反应,并增加机体的代谢功能障碍[21],以腹围增加为代表的腹部脂肪堆积患者表现出明显的WHR升高,无论BMI增加与否,都可能会对生育产生不利影响,相对于正常人更容易发生自然流产[22]。在IVF-ET相关研究中,WHR升高的肥胖女性相对于正常女性表现出妊娠率更低、流产率更高。对于接受辅助生殖助孕技术患者,针对WHR进行有效减脂后,同样可以降低妊娠不良事件的发生率[23-24]。

本研究另一项重要发现为TT水平的降低与RPL有关。绝经前女性的TT主要由卵巢分泌,随着年龄增长伴随出现的卵巢功能减退,睾酮水平也逐步下降,为了排除两组之间年龄差异的影响,本研究采用了多因素logistic回归矫正了其他因素后,TT水平的降低仍然是RPL的危险因素(OR= 0.59, 95%CI: 0.38 ~ 0.93, P= 0.023)。已有研究发现,在正常早期妊娠阶段,血清孕酮、雌二醇和睾酮水平显著升高,但游离睾酮比(游离睾酮/TT)水平显著下降[25]。遗憾的是,由于游离睾酮的测量方法昂贵耗时,本研究中对照组女性并未进行游离睾酮测定,因此未能进行两组间游离睾酮的比较。既往有研究提示,稽留流产的女性较正常妊娠女性TT下降,游离睾酮升高[26]。正常妊娠时性激素结合球蛋白(sex hormone binding globulin, SHBG)和雌二醇之间存在正相关关系,SHBG 的产生随着雌激素的增加而平行增加,而游离睾酮下降。美国的一项横断面研究曾分析61名孕妇整个妊娠期间TT和游离睾酮的变化, 58名女性中TT水平随孕周增加出现上升,而TT值未升高的3名孕妇,随后有2名发生了早期流产,提示了妊娠早期TT水平的上升对于妊娠维持的重要意义[27]。这可能是由于雄激素可以诱导早期卵泡发育,影响卵泡

的募集与生长, 改善卵子质量。并且孕期的雄激素可以协同孕激素参与细胞周期调节, 使子宫内膜蜕膜化, 有利于胚胎着床, 已有研究证明, 雄激素可以通过提高卵巢刺激反应, 增加获卵数, 并改善妊娠结局[28], 因此雄激素现常被用于改善IVF-ET中卵巢反应低下患者的卵巢反应。对于正常妊娠女性而言, 雄激素直接调节妊娠期间免疫耐受, 以保证妊娠的正常进行和分娩正常发动, 当血清睾酮随年龄增长逐渐下降时, 窦卵泡数的减少和AMH的下降也会平行出现, 对不良妊娠结局的发生有推动作用[29], 适量补充雄激素可显著增加AMH水平, 从而改善卵巢功能。但雄激素过高, 也可能会导致流产[30], 睾酮可以通过影响子宫颈组织的重构和子宫肌层平滑肌纤维的成熟, 促进子宫颈内胶原纤维不断减少, 使宫颈成熟软化扩张, 增加自然流产概率。 高雄激素环境可影响内膜生长与胚胎植入相关蛋白, 可能导致子宫内膜容受性损伤和RPL的发生[31]。因此不同类型的雄激素如TT及游离睾酮对早期妊娠的影响及在妊娠维持中的具体作用机制有待进一步的深入研究。

此外本研究同样发现高龄会增加RPL的风险, 高龄被认为是不良妊娠结局的重要危险因素[32] , 这可能是由于随着孕妇年龄的增大, 更容易出现胰腺β细胞功能障碍、胰岛素敏感性恶化和脂质代谢紊乱[33], 也更容易出现心功能储备下降, 从而导致胎盘灌注不足, 引发不良妊娠结局。自2016年中国实施全面两孩政策后, 预期高龄孕产妇比例将增加, 如何提高高龄孕产妇的优生优育, 将是我们未来面临的一个潜在挑战。

本研究也存在一定的局限性, 本研究重点围绕年龄、BMI、WHR及常规辅助生殖助孕相关的实验室指标展开, 未将妊娠期女性的环境暴露因素、孕期抗生素的使用、孕妇的心理健康等因素纳入分析中。且由于条件受限, 未能将游离睾酮、SHBG等重要的性激素纳入分析中。同时, 本研究为回顾性病例对照研究, 两组间基线如年龄、BMI、WHR存在差异, 尽管通过多因素logistic回归进行了校正, 但未来仍需开展严密设计、良好匹配的前瞻性研究进一步探索这些因素与RPL之间的关系。

综上, 在接受IVF-ET的患者中, 高龄、TT水平的降低、WHR升高及FINS水平升高是反复妊娠丢失的危险因素。TT水平与自然流产次数间存在线性相关关系, 随着流产次数的增加, TT呈现出下降趋势。

*　　*　　*

作者贡献声明　曹亚聪负责论文构思、初稿写作和审读与编辑写作, 李一鸣负责论文构思和初稿写作, 潘萍和杜涛负责调查研究, 杨冬梓负责论文构思, 赵晓苗负责论文构思、经费获取和研究项目管理。所有作者已经同意将文章提交给本刊, 且对将要发表的版本进行最终定稿, 并同意对工作的所有方面负责。

Author Contribution　CAO Yacong is responsible for conceptualization, writing--original draft, and writing--review and editing. LI Yiming is responsible for conceptualization and writing--original draft. PAN Ping and DU Tao are responsible for investigation. YANG Dongzi is responsible for conceptualization. ZHAO Xiaomiao is responsible for conceptualization, funding acquisition, and project administration. All authors consented to the submission of the article to the Journal. All authors approved the final version to be published and agreed to take responsibility for all aspects of the work.

利益冲突　所有作者均声明不存在利益冲突

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参　考　文　献

[1] DIMITRIADIS E, MENKHORST E, SAITO S, *et al*. Recurrent pregnancy loss. Nat Rev Dis Primers, 2020, 6(1): 97. doi:10.1038/s41572-020-00238-x.

[2] QUENBY S, GALLOS I D, DHILLON-SMITH R K, *et al*. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. Lancet, 2021, 397(10285): 1658–1667. doi: 10.1016/s0140-6736(21)00682-6.

[3] LIN Z, LI Q, SUN Y, *et al*. Interactions between genetic variants involved in the folate metabolic pathway and serum lipid, homocysteine levels on the risk of recurrent spontaneous abortion. Lipids Health Dis, 2019, 18(1): 143. doi: 10.1186/s12944-019-1083-7.

[4] LI D, ZHENG L W, ZHAO D H, *et al*. The role of immune cells in recurrent spontaneous abortion. Reprod Sci, 2021, 28(12): 3303–3315. doi: 10.1007/s43032-021-00599-y.

[5] SALEHI A M, GHAFOURIAN M, AMARI A, *et al*. Evaluation of $CD3^+$ T cell percentage, function and its relationship with serum vitamin D levels in women with recurrent spontaneous abortion and recurrent implantation failure. Iran J Immunol, 2022, 19(4): 369–377. doi: 10.22034/iji.2022.91464.2083.

[6] 中华医学会妇产科学分会产科学组, 复发性流产诊治专家共识编写组. 复发性流产诊治专家共识(2022). 中华妇产科杂志, 2022, 57(9): 653–667. doi: 10.3760/cma.j.cn112141-20220421-00259.
Obstetrics Subgroup, Chinese Society of Obstetrics and Gynecology; Chinese Medical Association, Chinese Expert Consensus Group on Diagnosis and Management of Recurrent Spontaneous Abortion. Chinese expert consensus on diagnosis and management of recurrent spontaneous abortion (2022). Chin J Obstet Gynecol, 2022, 57(9): 653–667. doi: 10.3760/cma.j.cn112141-20220421-00259.

[7] COUGHLAN C, LEDGER W, WANG Q, *et al*. Recurrent implantation failure: definition and management. Reprod Biomed Online, 2014, 28(1): 14–38. doi: 10.1016/j.rbmo.2013.08.011.

[8] LIN C A, LIU Y P, CHEN Y C, *et al*. Gender-specific and age-specific associations of the homoeostasis model assessment for IR (HOMA-IR) with albuminuria and renal function impairment: a retrospective cross-sectional study in Southeast China. BMJ Open, 2021, 11(12): e053649. doi: 10.1136/bmjopen-2021-053649.

[9] WANG T, LU J, SHI L, *et al*. Association of insulin resistance and β-cell dysfunction with incident diabetes among adults in China: a nationwide,

population-based, prospective cohort study. Lancet Diabetes Endocrinol, 2020, 8(2): 115–124. doi: 10.1016/s2213-8587(19)30425-5.

[10] CHEN X, YANG D, LI L, *et al*. Abnormal glucose tolerance in Chinese women with polycystic ovary syndrome. Hum Reprod, 2006, 21(8): 2027–2032. doi: 10.1093/humrep/del142.

[11] ZHANG G M, GU Y Y. Diagnostic value of Procalcitonin, C-reactive protein-to-lymphocyte ratio (CLR), C-reactive protein and neutrophil-to-lymphocyte ratio (NLR) for predicting patients with Bacteraemia in the intensive care unit. J Crit Care, 2024, 81: 154538. doi: 10.1016/j.jcrc.2024.154538.

[12] WALSH S W, NUGENT W H, Al DULAIMI M, *et al*. Proteases activate pregnancy neutrophils by a protease-activated receptor 1 pathway: epigenetic implications for preeclampsia. Reprod Sci, 2020, 27(11): 2115–2127. doi: 10.1007/s43032-020-00232-4.

[13] GAO P, ZHA Y, WEI L, *et al*. G-CSF: a vehicle for communication between trophoblasts and macrophages which may cause problems in recurrent spontaneous abortion. Placenta, 2022, 121: 164–172. doi: 10.1016/j.placenta.2022.03.125.

[14] SCARPELLINI F, KLINGER F G, ROSSI G, *et al*. Immunohistochemical study on the expression of G-CSF, G-CSFR, VEGF, VEGFR-1, Foxp3 in first trimester trophoblast of recurrent pregnancy loss in pregnancies treated with G-CSF and controls. Int J Mol Sci, 2020, 21(1): 285. doi: 10.3390/ijms21010285.

[15] BERT S, WARD E J, NADKARNI S. Neutrophils in pregnancy: new insights into innate and adaptive immune regulation. Immunology, 2021, 164(4): 665–676. doi: 10.1111/imm.13392.

[16] KUPPUSWAMI J, SENTHILKUMAR G P. Nutri-stress, mitochondrial dysfunction, and insulin resistance-role of heat shock proteins. Cell Stress Chaperones, 2023, 28(1): 35–48. doi: 10.1007/s12192-022-01314-9.

[17] CAI W Y, LUO X, LV H Y, *et al*. Insulin resistance in women with recurrent miscarriage: a systematic review and meta-analysis. BMC Pregnancy Childbirth, 2022, 22(1): 916. doi: 10.1186/s12884-022-05256-z.

[18] HANTOUSHZADEH S, KOHANDEL GARGARI O, SHAFIEE A, *et al*. Glucose metabolism tests and recurrent pregnancy loss: evidence from a systematic review and meta-analysis. Diabetol Metab Syndr, 2023, 15(1): 3. doi:10.1186/s13098-022-00973-z.

[19] 叶春雨, 陈秀荣, 邱凌钰, 等. 胰岛素抵抗与复发性流产相关性研究进展. 中国实用妇科与产科杂志, 2022, 38(2): 243–246. doi: 10.19538/j.fk2022020127.

YE C Y, CHEN X R, QIU L Y, *et al*. Research progress on the correlation between insulin resistance and recurrent abortion. Chin J Pract Gynecol Obstet, 2022, 38(2): 243–246. doi: 10.19538/j.fk2022020127.

[20] XU H, JIN C D, GUAN Q B. Causal effects of overall and abdominal obesity on insulin resistance and the risk of type 2 diabetes mellitus: a two-sample mendelian randomization study. Front Genet, 2020, 11: 603. doi: 10.3389/fgene.2020.00603.

[21] BLUM M R, POPAT R A, NAGY A, *et al*. Using metabolic markers to identify insulin resistance in premenopausal women with and without polycystic ovary syndrome. J Endocrinol Invest, 2021, 44(10): 2123–2130. doi: 10.1007/s40618-020-01430-2.

[22] BU Z, HU L, SU Y, *et al*. Factors related to early spontaneous miscarriage during IVF/ICSI treatment: an analysis of 21, 485 clinical pregnancies. Reprod Biomed Online, 2020, 40(2): 201–206. doi: 10.1016/j.rbmo.2019.11.001.

[23] SILVESTRIS E, De PERGOLA G, ROSANIA R, *et al*. Obesity as disruptor of the female fertility. Reprod Biol Endocrinol, 2018, 16(1): 22. doi: 10.1186/s12958-018-0336-z.

[24] LI M C, MINGUEZ-ALARCON L, ARVIZU M, *et al*. Waist circumference in relation to outcomes of infertility treatment with assisted reproductive technologies. Am J Obstet Gynecol, 2019, 220(6): 578.e1–578.e13. doi: 10.1016/j.ajog.2019.02.013.

[25] WITT B R, WOLF G C, WAINWRIGHT C J, *et al*. Relaxin, CA-125, progesterone, estradiol, Schwangerschaft protein, and human chorionic gonadotropin as predictors of outcome in threatened and nonthreatened pregnancies. Fertil Steril, 1990, 53(6): 1029–1036. doi: 10.1016/S0015-0282(16)53580-8.

[26] TAKEUCHI T, NISHII O, OKAMURA T, *et al*. Free testosterone and abortion in early pregnancy. Int J Gynaecol Obstet, 1993, 43(2): 151–156. doi: 10.1016/0020-7292(93)90322-n.

[27] BAMMANN B L, COULAM C B, JIANG N S. Total and free testosterone during pregnancy. Am J Obstet Gynecol, 1980, 137(3): 293–298. doi: 10.1016/0002-9378(80)90912-6.

[28] INNOCENTI F, FIORENTINO G, CIMADOMO D, *et al*. Maternal effect factors that contribute to oocytes developmental competence: an update. J Assist Reprod Genet, 2022, 39(4): 861–871. doi: 10.1007/s10815-022-02434-y.

[29] DILAVER N, PELLATT L, JAMESON E, *et al*. The regulation and signalling of anti-Müllerian hormone in human granulosa cells: relevance to polycystic ovary syndrome. Hum Reprod, 2019, 34(12): 2467–2479. doi: 10.1093/humrep/dez214.

[30] ZHANG Y, ZHAO W, XU H, *et al*. Hyperandrogenism and insulin resistance-induced fetal loss: evidence for placental mitochondrial abnormalities and elevated reactive oxygen species production in pregnant rats that mimic the clinical features of polycystic ovary syndrome. J Physiol, 2019, 597(15): 3927–3950. doi: 10.1113/jp277879.

[31] RAHMAN T U, ULLAH K, GUO M X, *et al*. Androgen-induced alterations in endometrial proteins crucial in recurrent miscarriages. Oncotarget, 2018, 9(37): 24627–24641. doi: 10.18632/oncotarget.24821.

[32] HUANG C, JIANG Q, SU W, *et al*. Age-specific effects on adverse pregnancy outcomes vary by maternal characteristics: a population-based retrospective study in Xiamen, China. BMC Public Health, 2023, 23(1): 326. doi: 10.1186/s12889-023-15235-4.

[33] SHAN D, QIU P Y, WU Y X, *et al*. Pregnancy outcomes in women of advanced maternal age: a retrospective cohort study from China. Sci Rep, 2018, 8(1): 12239. doi: 10.1038/s41598-018-29889-3.

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