刁飞扬, 南京医科大学第一附属医院(江苏省人民医院、江苏省妇幼保健院)生殖医学中心主任。现任国家辅助生殖技术准入评审专家, 江苏省卫健委生殖医学创新中心主任, 江苏省医学会生殖医学分会副主委。美国匹兹堡大学医学中心/MWRI研究所生殖遗传访问学者。在排卵障碍性疾病、子宫内膜容受性、辅助生殖技术安全性和生殖遗传等方向获多项国家自然科学基金、国家重点研发计划等资助。在*J Clin Invest*、*Am J Hum Genet*、*Cell Res*、*Lancet Regional Health*等杂志发表论文多篇。

子宫内膜与辅助生殖技术——从基础研究到临床转化

李凤[1,2] 张园[2] 王琳[2] 刁飞扬[1,2]*

([1]南京医科大学生殖医学与子代健康全国重点实验室, 南京 211166;
[2]南京医科大学第一附属医院生殖医学中心, 南京 210029)

摘要 经过四十多年的发展, 辅助生殖技术已成为不孕不育的有效治疗手段。子宫内膜作为“土壤”, 其正常的结构和功能是胚胎成功植入、健康发育的关键因素。子宫内膜容受性成为近年来生殖医学领域的研究热点, 基础研究相应地极大促进了临床诊断流程和治疗路径的迭代。该文从子宫内膜在周期性修复、微生态平衡和胚胎着床中的生理功能出发, 聚焦损伤、感染、非生理水平激素影响辅助生殖妊娠结局的病理因素及其相应诊疗措施, 关注与子宫内膜相关的辅助生殖后妊娠期并发症和子代健康, 从子宫内膜的基础研究到临床转化的角度, 探讨进一步提高辅助生殖技术有效性和安全性的新角度和新方法。

关键词 辅助生殖技术; 子宫内膜损伤; 子宫内膜生态失调; 子宫内膜容受性; 妊娠期并发症; 围产期结局

Endometrium and Assisted Reproductive Rechnology — from Basic Research to Clinical Translation

LI Feng[1,2], ZHANG Yuan[2], WANG Lin[2], DIAO Feiyang[1,2]*

([1]*State Key Laboratory of Reproductive Medicine and Offspring Health, Nanjing Medical University, Nanjing 211166, China*;
[2]*Clinical Center of Reproductive Medicine, the First Affiliated Hospital with Nanjing Medical University, Nanjing 210029, China*)

Abstract ART (assisted reproductive technology) has evolved significantly over the past four decades, establishing itself as a potent solution for infertility. The endometrium, often referred as the ‘soil’, is instrumental in ensuring successful embryo implantation and healthy development. Recent years have seen a surge in research interest towards endometrial receptivity within the sphere of reproductive medicine. This interest, coupled with rel-

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*通信作者。Tel: 025-68302222, E-mail: diaofeiyang@njmu.edu.cn
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*Corresponding author. Tel: +86-25-68302222, E-mail: diaofeiyang@njmu.edu.cn

evant basic research, has substantially advanced the evolution of clinical diagnostic procedures and treatment pathways. This paper delves into the physiological roles of the endometrium in cyclic repair, microbiota equilibrium, and embryo implantation. It underscores pathological factors, including injury, endometrial dysbiosis, and non-physiological hormonal levels, that potentially influence the outcomes of ART pregnancies, and discusses the associated diagnostic and therapeutic measures. The paper also sheds light on post-ART complications and offspring health related to the endometrium. By bridging the gap between basic endometrial research and clinical application, this paper aims to explore innovative perspectives and methodologies to further enhance the efficacy and safety of assisted reproductive technology.

Keywords ART (assisted reproductive technology); endometrium injury; endometrial dysbiosis; endometrial receptivity; pregnancy complication; perinatal outcome

自1978年第一例试管婴儿诞生以来, 辅助生殖技术(assisted reproductive technology, ART)取得了快速发展, 临床妊娠率由最初的6%上升至47%~57%[1-2]。胚胎质量、子宫内膜容受性、胚胎与子宫内膜同步发展是影响移植后妊娠结局的三大关键因素, 其中子宫内膜因素约占2/3[3-4]。近年来, 控制性卵巢刺激和胚胎体外培养技术的优化及二代测序技术的应用, 极大地促进了整倍体囊胚的获得, 但是部分患者移植整倍体囊胚后仍然出现反复着床失败[5]。对早期妊娠丢失患者流产物的研究也提示仅有53.1%的患者存在染色体核型的异常[6]。子宫内膜对早期胚胎发育对妊娠并发症和子代健康的影响也时见报道, 这使之受到越来越多学者的关注。本文从子宫内膜在周期性修复、微生态平衡和胚胎着床中的生理功能出发, 对影响辅助生殖妊娠结局的子宫内膜病理状态及其临床处理, 及其对辅助生殖后妊娠期并发症和子代健康相关研究作一系统综述。

1 子宫内膜的生理功能

1.1 子宫内膜的生理性修复

子宫内膜特有双层结构包括表面的功能层和底部的基底层, 是其高度再生修复能力的结构基础。功能层和基底层共同合作, 实现子宫内膜的周期性修复、增殖和分泌期转化, 以便为胚胎着床提供适宜的“土壤”。

功能层由腺体组织和结缔组织构成。在月经期, 功能层原有的上皮细胞发生激素撤退性脱落, 腺体结构重塑, 周围基质中子宫内膜干细胞分化成新的上皮细胞, 此为非激素依赖性修复阶段。在增殖期及分化期, 子宫内膜将随着体内雌、孕激素的序贯变化进行程序化修复, 即通过上皮、间质细胞的增殖和分化, 由成纤维细胞重塑细胞外基质, 重建内膜血管, 进而达到完全无痕化修复, 此为激素依赖性修复阶段[7]。

基底层则主要由基质构成, 在月经期几乎不发生脱落, 不受卵巢激素影响, 但其结构和功能的完整性对内膜修复至关重要, 在每个月经周期增殖分化为新的子宫内膜功能层。

1.2 子宫内膜微生态

子宫腔曾被认为是无菌的, 然而近年来的研究证实子宫腔内存在特殊的低生物量微生物组。正常情况下, 共生微生物与子宫内膜上皮细胞作用后可以触发各种抗菌肽释放到子宫腔, 同时维持上皮屏障的稳定, 对抗病原体入侵。异常的致病菌则可能导致子宫内膜防御功能受损, 进而引起子宫内膜炎、着床失败乃至不孕的发生。

最近的一项多中心前瞻观察性研究发现, 乳酸杆菌是子宫内膜组织和子宫内膜液中最丰富的菌属, 而其他多种细菌, 如厌氧球菌、奇异菌属、双歧杆菌和加德诺菌属等, 也有较低的比例。乳酸杆菌与加德诺菌属、双歧杆菌和奇异菌属呈负相关, 与共生菌梭菌和链霉菌呈正相关。研究人员对342例无症状感染的不孕患者宫腔液和子宫内膜组织样本进行16S rRNA基因测序分析发现, 活产组子宫内膜乳杆菌丰度最高, 子宫内膜微生物组呈失调状态且与着床失败相关, 提示胚胎移植前子宫内膜微生态是预测辅助生殖妊娠结局的重要生物标志物之一, 为改善生殖结局提供新的诊疗切入点[8]。

子宫内膜微生态对妊娠结局的影响机制尚处在探索阶段。已知月经周期中微生物丰度存在周期性变化, 母–胎界面存在特殊的子宫自然杀伤细胞(uterine natural killer, uNK)和特定亚群T细胞网络。研究发现一方面子宫内膜局部免疫细胞(uNK细胞、

T细胞以及巨噬细胞等)分泌的各种免疫分子和细胞因子在维持共生微生物的耐受性方面具有重要调节作用; 另一方面共生微生物可在细胞水平影响子宫内膜中固有免疫和适应性免疫系统。因此, 子宫内膜局部低生物量微生物或能通过调节子宫局部免疫系统功能, 改变子宫内膜容受性, 影响早期胚胎植入和胎盘形成, 进一步影响女性生育能力、妊娠并发症和宫内胎儿发育[9-10]。

1.3 着床窗口期子宫内膜与胚胎的互作

人类胚胎在排卵后4天进入子宫腔, 同时子宫内膜经历增生期到分泌期转化, 以及相关血管和免疫反应的一系列变化, 在排卵后6~8天具备短暂的接受胚胎着床能力, 准备接受囊胚着床, 这段时间被称为“种植窗”(window of implantation, WOI)。

胚胎的黏附和植入依赖子宫内膜无菌性炎症状态, 但随后长期的妊娠维持又需要内膜转为炎症抑制状态, 其机制包括延长卵巢孕酮产生, 控制胎盘植入, 建立免疫耐受和子宫内膜发生蜕膜化转变[11]。蜕膜化使子宫内膜间质发生遗传、形态、代谢、生化、血管和免疫等一系列变化[12]。子宫内膜基质细胞(endometrial stromal cells, ESCs)分化为蜕膜基质细胞(deciduastromal cells, DSCs)后可产生催乳素(prolactin, PRL)和胰岛素样生长因子结合蛋白1(insulin-like growth factor binding 1, IGFBP1)等蜕膜化的独特生物标记物[12-13]。ESCs产生的IL-15是调节人uNK 介导的细胞分化、免疫耐受功能的重要调节因子, 而uNK是子宫内膜中主要的白细胞, 在调节母体免疫耐受、介导着床方面具有重要作用[13]。早孕阶段, uNK聚集在螺旋动脉周围影响子宫内膜螺旋动脉重塑, 在妊娠期间母–胎界面子宫内膜血管生成方面发挥重要作用。蜕膜化是在整个子宫内膜重塑的背景下发生的一个广泛细胞重编程过程, 继而建立了一个能够促进胚胎着床、保护胚胎免受母体免疫攻击和促进胎盘发育的子宫内膜环境。越来越多的实验和临床证据有力地表明, 蜕膜化的缺陷或破坏可导致母–胎界面中的血管生成障碍和uNK细胞数量/功能的异常, 这可能导致生殖失败[12], 是复发性流产和反复种植失败(repeated implantation failure, RIF)的重要病理机制之一。

2 子宫内膜病理变化与辅助生殖妊娠结局

2.1 子宫内膜损伤的病理性修复

各种严重的病理性因素如宫腔不当操作、子宫内膜缺血、感染等因素均可造成子宫内膜损伤, 其特征是破坏子宫内膜基底层和子宫内膜血运, 引发炎症反应, 抑制子宫内膜再生。既往研究显示, 子宫内膜损伤后的病理性修复与纤维化的异常激活密切相关: 损伤后的子宫内膜上皮层修复障碍, 间质裸露导致纤维活性增加, 促纤维化细胞分泌增多, 成纤维细胞过度增生, 进而导致血管再生障碍和子宫内膜增殖障碍, 纤维细胞沉积形成瘢痕。宫腔瘢痕化破坏了内膜的血液供应和内肌层的完整性, 进而导致了蜕膜化异常, 深层滋养细胞浸润和血管纤维化受损后造成局部缺血和缺氧等不可逆变化。子宫内膜的病理性修复引发的子宫内膜纤维化和瘢痕形成可导致子宫内膜容受性下降, 进而造成胚胎移植后的着床率下降、流产率增加, 远期可能导致胎盘结构功能异常, 增加妊娠并发症和子代发育异常的风险[14-16]。

2.2 损伤

2.2.1 子宫内膜损伤的常见类型 子宫内膜损伤以宫腔粘连(intrauterine adhesion, IUA)和薄型子宫内膜(thin endometrium, TE)最为常见, 是子宫内膜病理性修复导致的常见后遗症, 会降低辅助生殖技术助孕的成功率, 增加胎盘发育不良导致的妊娠并发症发生率[17]。

宫腔粘连一般继发于各种宫腔操作导致的子宫内膜基底层细胞损伤, 表现为子宫内膜基底细胞数量减少、活力下降甚至发生凋亡, 子宫内膜上皮细胞无法完全修复导致萎缩和纤维化, 进而形成各种程度的宫腔粘连, 影响月经量、胚胎黏附及早期着床[18-19]。据统计, IUA在不孕症患者中的发病率为13%, 而8%的不孕症继发于IUA[15,20]。除了经典纤维化通路TGF-β1/Smad通路之外[21], 异常激活的Wnt/β-catenin通路也可促进TGF-β介导的子宫内膜纤维化[22]。FOXF2蛋白是Wnt/β-catenin通路的上游调控分子之一, 下调FOXF2的表达可抑制TGF-β1诱导的子宫内膜间质细胞纤维化和IUA的进展[23]。

子宫内膜厚度是简便易行的评价子宫内膜容受性的指标之一。目前临床上多将子宫内膜厚度<7 mm作为薄型子宫内膜诊断标准, 与≥7 mm的患者相比, 前者临床妊娠率显著降低, 持续妊娠率和活产率亦呈现降低趋势[24]。薄型子宫内膜病因非常复杂。口服避孕药、促性腺激素释放激素激动剂、氯米芬等药物治疗所导致的功能性薄型子宫内膜, 大多在停药后恢复。结核等感染诱发的子宫内膜炎、

反复宫腔操作造成的损伤等可能造成子宫内膜基底层和血管受损、子宫内膜修复障碍，诱导形成器质性薄型子宫内膜，严重时会形成不可逆的顽固性薄型子宫内膜。MIWA等[17]早在2009年就描述了薄型子宫内膜的病理特点：子宫血流阻抗增高导致腺上皮生长不良，血管内皮生长因子表达水平降低导致血管发育不良，进而导致子宫内膜局部血供和氧供不足。在薄型子宫内膜的增殖期，细胞衰老、胶原过度沉积以及细胞增殖相关基因表达受损均可以导致子宫内膜生长缺陷，局部巨噬细胞和自然杀伤细胞(natural killer, NK)浓度过低也会进一步阻碍子宫内膜修复[25]。

2.2.2 子宫内膜损伤的治疗 宫腔镜及药物治疗。宫腔镜下宫腔粘连松解术是治疗IUA的主要手段，旨在重建子宫正常结构，降低粘连复发率，促进子宫内膜修复。重度宫腔粘连患者术后复发几率为20.0%~62.5%[26]。加快术后创面修复和内膜再生对预后至关重要，术中使用羊膜、宫腔球囊和宫内节育器，手术前后使用激素(如雌激素、他莫昔芬等)和改善子宫内膜血流灌注的药物(如低剂量阿司匹林、西地那非等)。

对于重度IUA和薄型子宫内膜患者，药物联合宫腔镜手术治疗效果欠佳，因此迫切需要探索新的方法来改善子宫内膜的修复和再生。因此以细胞治疗为基础发展出多种新型辅助促进内膜细胞增殖或再生的治疗手段，如干细胞(stem cells, SCs)、富血小板血浆(palette-rich plasma, PRP)、粒细胞集落刺激因子(granulocyte colony stimulating factor, G-CSF)、生长激素(growth hormone, GH)等逐渐应用于临床，旨在改善此类患者的妊娠结局。

2004年，CHEN等[27]首次证实子宫内膜中存在子宫内膜干/祖细胞(endometrial stem cells, enSCs)。子宫中存在少量的基质干细胞和上皮干细胞，在月经周期中促进子宫内膜增殖，但随着子宫损伤而数量减少[28]。与健康女性相比，IUA患者子宫内膜干细胞的比例和迁移、侵袭能力均显著降低，间接影响损伤子宫内膜的自我修复[29]。因此使用具有很强自我更新能力和多向增殖分化能力的干细胞促进子宫内膜再生增殖，成为内膜损伤的重要手段。从骨髓、脂肪组织、脐带血、月经血、羊膜等组织中分离得到的间充质干细胞(mesenchymal stem cells, MSCs)应用最为广泛，已初步应用于临床。

(1) 干细胞治疗。骨髓间充质干细胞(bone marrow mesenchymal stem cells, BM-MSCs)易于体外培养，是研究最早、目前应用最广泛的干细胞类型，可以分化为多种非造血细胞，但有报道称其具有一定的致瘤性[30]。BM-MSCs在修复损伤组织中不仅通过细胞分化发挥重要作用，同时也可通过旁分泌路径分泌VEFG、TNF-α、基质金属蛋白酶、B淋巴细胞瘤-2、成纤维细胞生长因子-b(fibroblast growth factor-b, b-FGF)、IL-6等细胞因子发挥促血管生成、抗炎、抗纤维化、抗细胞凋亡等作用，促进子宫内膜再生，改善容受性、提高妊娠率[26]。BMSCs联合雌激素还可以通过Wnt/β-catenin信号通路协同促进子宫内膜再生，抑制上皮–间质转化(epithelial-mesenchymal transition, EMT)进程，促进子宫内膜修复[31-32]。

脂肪间充质干细胞(adipose-derived mesenchymal stem cell, AD-MSC)较BM-MSC具有较高的增殖、分泌和抗衰老能力，更易从自体获取，但仍需要具有整形外科的技术条件。对薄型子宫内膜和子宫内膜损伤模型大鼠的研究提示：AD-MSC负载于胶原内膜补片可使子宫内膜LIF、TGF-β和FGF2表达上调，子宫内膜增殖能力和宫腔上皮面积增加[33]；AD-MSC负载于胶原支架复合物可促进子宫内膜腺体增生和血管生成，增强免疫调节和抗纤维化作用[34]。

人羊膜间充质干细胞(human amniotic mesenchymal stem cells, hAMSCs)和脐带间充质干细胞(umbilical cord-derived mesenchymal stem cells, UC-MSCs)具有安全性高、免疫原性低、增殖能力强、易于收集的优点，较多应用于临床研究。hAMSCs移植联合雌激素治疗宫腔粘连大鼠，增强子宫内膜腺体增殖能力，减少内膜纤维化，血管内皮生长因子和细胞角蛋白的表达能力更强[35]。UC-MSCs治疗薄型子宫内膜大鼠后，增强了迁移到损伤部位的ESCs的增殖能力，通过Itga1和Thbs介导细胞外基质与受体相互作用通路参与内膜损伤修复，增加了子宫内膜厚度、腺体数量，可改善胚胎着床数[36]。在临床研究中，UC-MSCs改善了雌激素使用后子宫内膜厚度仍≤5.5 mm而取消冻融胚胎移植(frozen-thawed embryo transfer, FET)的薄型子宫内膜患者的妊娠率，免疫组化结果显示，UC-MSC通过上调Ki-67蛋白、雌激素受体α和孕激素受体表达促进子宫内膜血管生成、增殖和分化，改善了子宫内膜对激素的生物

学反应[37]。

虽然干细胞来源及制备方式存在区别, 但已有临床试验显示其对内膜生长及妊娠结局改善的效果优于传统治疗, 将干细胞与生物材料支架相结合用于治疗子宫内膜损伤, 能更好地体现其优势[38-40]。

(2) 外泌体(mesenchymal stem cells derived exosome, MSC-exo)。由于干细胞获取复杂、归巢率低和体内寿命短, MSCs来源细胞外囊泡特别是外泌体因其来源丰富、免疫原性低、致瘤性低等优点在临床研究中获得重视[41]。既往研究发现间充质干细胞来源的MSC-exo可以通过TGF-β1/smad通路逆转EMT, 促进受损子宫内膜的修复, 它们通过改变信号通路、传递miRNA、释放细胞因子或改变功能蛋白发挥作用。最新一项研究发现外泌体可通过递送miR-125b-5p、miR-30c-5p和miR-23a-3p, 从而抑制smad2和smad3的表达, 阻断TGF-β/smad通路, 阐明了MSC-exo特异性miRNAs对IUA有治疗作用的潜在分子机制[42]。另外, 外泌体还参与机体免疫系统, 从而调节受损组织的炎症反应来修复子宫内膜, 脂肪间充质干细胞来源的外泌体通过增强整合素β3、LIF和VEGF的表达, 促进子宫内膜再生和胶原重塑, 从而恢复IUA大鼠模型的生育能力[43], 另外, 还可通过UBR4促进YAP泛素化降解和促进YAP核质转位减轻子宫内膜纤维化[44]; 还有研究发现PRP中含有的生长因子PDGFBB可通过AKT/NF-κB信号通路改善MenSCs的活力、迁移和干性, 还可以通过抑制YAP活性扩增MenSCs来源的外泌体来减轻子宫内膜纤维化[45]。

特洛细胞(telocyte, TC)是一种新型的间质细胞, 存在于女性子宫中, 在蜕膜化缺陷的妇科疾病中具有体外治疗潜力。宫腔粘连的小鼠研究显示TC来源的外泌体可通过减轻纤维化、增强MET和血管生成, 有效促进子宫内膜的修复和再生[46]。

目前, 外泌体可从多种干细胞, 包括骨髓、脂肪、经血、胎盘等中提取, 在子宫内膜损伤的动物实验中取得良好的治疗效果, 但是缺少在临床上的相关应用。随着培养技术的发展和日趋成熟, 相信将来, 有望利用外泌体为IUA的精准治疗提供新的视角。

(3) 宫腔灌注治疗。PRP中血小板含量是正常血浆的3~5倍, 活化后的血小板诱导α颗粒释放一系列生长因子如血管内皮生长因子、转化生长因子、血小板源性生长因子、胰岛素样生长因子、成纤维细胞生长因子、表皮生长因子等。与普通血浆相比, PRP中PDGFBB和血管内皮生长因子的水平升高, 增强了子宫内膜细胞增殖和血管生成[47]。

PRP自体血来源避免了异体血的免疫原性及经血液传播疾病的风险, 使其临床可用性增加[39]。近年来小鼠模型研究PRP可减轻纤维化程度, 促进子宫内膜修复, 其通路可能是血小板诱导释放的生长因子诱导子宫内膜间充质干细胞(endometrial mesenchymal stem cells, EnMSCs)的迁移和增殖, 进而分化为子宫内膜细胞[48], 增加应激诱导蛋白p53和胞外酶MMP9的产生, 加速子宫内膜修复[49]; 可以提高小鼠模型和薄型子宫内膜患者的妊娠率和活产率[50-51], 降低宫腔粘连患者术后的复发率[52]。但因临床研究时间不长, PRP的制备、应用指征、治疗方案仍需进一步探讨。

骨髓细胞、基质细胞、成纤维细胞和巨噬细胞产生的G-CSF是一种糖蛋白, 具有生长因子和细胞因子的功能, 在内皮细胞、胎盘细胞、滋养层细胞和颗粒黄体细胞表面表达有其受体。研究表明, G-CSF有助于干细胞的动员、迁移和分化, 同时还可通过促进血管生成促进子宫内膜再生, 并通过降低凋亡活性减少细胞死亡[53]。2013年首次报道G-CSF应用于难治性薄型子宫内膜, 治疗后4例患者在试管婴儿助孕中均获得了成功妊娠[54]。在宫腔镜分离宫腔粘连放置球囊术后第7天宫腔灌注G-CSF可以改善患者子宫内膜厚度及累积妊娠率、活产率[55]。动物实验表明, G-CSF宫腔灌注可增加VEGF和LIF的表达水平, 刺激子宫内膜增殖和血管生成, 改善薄型子宫内膜大鼠模型的子宫内膜容受性[56]。最近一项G-CSF对IVF不孕症疗效的系统评价和Meta分析表明G-CSF可以增加薄型子宫内膜IVF患者的临床妊娠率和子宫内膜厚度[57]。

GH是一种由脑腺垂体分泌的, 能促进骨质和蛋白质合成、增加脂肪分解的激素。研究证实GH及其受体在卵巢和子宫组织中均有表达, 可改善卵巢低反应和反复种植失败的患者中卵母细胞质量提高临床妊娠率。研究表明, 生长激素可调节子宫内膜细胞的增殖和代谢, 分布在子宫内膜细胞上的 IGF可与GH结合, 通过旁分泌及自分泌机制在子宫内膜局部起作用[58]。动物实验表明宫腔灌注GH效果优于皮下注射, 可促进薄型子宫内膜大鼠模型的内膜

再生和修复[59], 改善辅助生殖助孕人群中TE患者的子宫内膜厚度和容受性, 从而改善妊娠结局[60]。

(4) 仿生电刺激(biomimetic electrostimulation, BES)疗法。目前认为仿生电刺激可能通过刺激子宫平滑肌收缩, 加快血流速度, 降低血管阻力, 从而增加子宫内膜厚度, 改善子宫内膜容受性。

一项动物实验表明仿生电刺激通过激活基质细胞衍生因子-1/CXC趋化因子受体4(SDF-1/CXCR4)通路促进移植BMSCs向损伤的子宫内膜迁移; 联合BM-MSCs治疗还可使子宫内膜细胞角蛋白和波形蛋白表达上调, 子宫内膜病变处VEGF和bFGF分泌增加, 可提高胚胎种植率[61]。

2022年QI等[62]将拟行激素替代周期FET的TE患者随机分为治疗组和对照组, 治疗组加用血管腔内理疗联合针灸治疗可显著增加薄型子宫内膜患者子宫内膜厚度及A型内膜比例, 改善了子宫内膜血流阻力。研究还发现薄型子宫内膜患者子宫内膜存在能量代谢异常, 影响自噬过程, 导致薄型子宫内膜容受性下降, 腔内理疗联合针刺介导AMPK/mTOR通路改善能量代谢, 促进自噬过程, 上调子宫内膜容受性相关HOXA10基因和蛋白的表达, 提高胚胎着床率和临床妊娠率。

目前BES促进子宫内膜生长及改善子宫内膜容受性的机制研究较少, 需进一步研究提供更多证据。

2.3 感染

2.3.1 子宫内膜炎 慢性子宫内膜炎(chronic endometritis, CE)是一种以子宫内膜间质区异常浆细胞浸润为特征的局部炎症性疾病, 通常呈无症状感染, 也可表现为不规则子宫出血、子宫内膜息肉、盆腔疼痛和白带量/性状异常等。诊断CE的金标准为子宫内膜活检后组织病理学分析, 并使用CD138抗体对浆细胞进行免疫组织化学染色。目前对CE的中浆细胞的诊断标准没有达成共识, 大多数学者认为在子宫内膜间质中存在多个(两个或两个以上)浆细胞是确认CE的必要条件, 因为诊断标准不统一, 报道的育龄女性CE的患病率从8%到72%不等[63]。据统计, 在27.4%的不孕妇女中, 子宫内膜息肉与CE之间存在关联, 子宫内膜息肉可能在没有感染的情况下机械地诱导宫腔慢性炎症, 而持续的炎症反应和感染加剧了子宫内膜息肉的发展, 但是宫内炎症是否是子宫内膜息肉发生的原因或结果尚不清楚[64]。

一项回顾性队列研究发现, 在患有子宫内膜息肉的不孕女性中, CE患病率高于可生育女性, 但接受CE治疗的不孕妇女的妊娠结局与未接受CE治疗的不孕妇女相似[65]。其他研究也发现, 宫腔镜术后口服抗生素治疗并不能改善CE患者的临床妊娠率[66]。但一项Mate分析表明在具有CE和反复种植失败病史的不孕妇女中使用抗生素治疗后改善了胚胎移植周期的妊娠结局[67]。LIU等[68]通过检测围着床期子宫内膜组织中缺氧诱导因子1a(hypoxiainducible factor 1a, HIF1a)、血管内皮生长因子A(vascular endothelial growth factor A, VEGFA)和血管内皮生长因子受体2(vascular endothelial growth factor receptor 2, VEGFR2)的表达水平, 发现$CD138^+$≥5个/10 HPFs的24例患者HIF1a、VEGFA和VEGFR2的蛋白和mRNA水平均显著升高(P<0.05), 且抗生素使用后子宫内膜HIF1a和VEGFA表达水平降低, 提示CE不孕妇女围着床期子宫内膜HIF1a的过度表达和过度血管化, 可能与子宫内膜容受性受损有关。最近的一项研究发现RIF患者和CE患者口服抗生素(多西环素和甲硝唑)联合宫腔灌注(庆大霉素和地塞米松)治疗后有较高的胚胎种植率、临床妊娠率和活产率, 此方法作为CE的一种新的治疗方法, 与单纯口服抗生素相比, 可以改善临床妊娠结局[69]。但最近的一篇Cochrane Meta分析纳入了两项RCT研究, 提示预防性抗生素治疗是否提高辅助生殖助孕人群的临床妊娠率和活产率的证据仍欠充分[70]。

2.3.2 子宫内膜微生态失衡 16S rRNA测序技术将CE的免疫组化诊断进一步扩展到了微生物群。子宫内膜微生物群包括正常共生微生物群和异常的致病微生物群。子宫内膜微生物组成在胚胎围着床期处于高度稳定的状态。一旦宫腔微生物群组成失衡, 则会使非乳酸杆菌为主的其他菌种增加, 引起子宫内膜炎症、着床失败和不孕的发生[71]。

研究显示CE患者子宫内膜微生物群的α多样性显著降低, 凋亡途径相关的促炎基因表达水平增加[72], 乳酸杆菌相对丰度显著降低[73]。非乳酸杆菌主导的子宫内膜微生物群(定义为<90%的乳酸杆菌)与辅助生殖技术着床、持续妊娠和活产率显著降低有关[74]。反复种植失败患者使用CE治疗后可改善辅助生殖助孕妊娠结局[75-76]。

子宫内膜微生态失衡导致不良妊娠结局的机制是目前研究的热点。微生态失衡引起的免疫应答和炎症反应及其之间的相互作用可能直接影响胚胎

着床的过程。研究显示, CE患者子宫内膜中$CD68^+$巨噬细胞、$CD83^+$成熟树突状细胞、$CD8^+$ T细胞和$Foxp3^+$ Treg细胞表达明显升高, 导致子宫内膜uNK细胞的动员、激活和成熟的异常[77]。利用高通量测序技术和16s rRNA测序技术, 分析CE引起的子宫内膜微生态失衡与RIF患者子宫内膜中免疫细胞的相互作用机制, 发现子宫内膜微生物群可通过干扰子宫内膜的碳水化合物代谢和/或脂肪代谢过程来调节免疫细胞发挥免疫调节作用, CE子宫内膜微生物可能通过脂多糖调节Th17反应及Th1/Th17比例, 降低子宫内膜容受性导致RIF的发生[78]。在动物实验中利用子宫组织代谢组学的相关性分析表明, 微生物群可通过影响子宫组织中重要代谢产物的表达水平调节微生物群的比例[79]。

目前微生态失衡的治疗方案主要为益生菌联用抗生素方案或合适的益生菌/益生元替代治疗方案, 手段单一, 疗效不确定。只有进一步阐明微生态失衡发生的机制, 才能够制定更有效的临床治疗方案, 改善子宫内膜微生态, 促进胚胎着床及改善妊娠结局。

2.4 激素

2.4.1 *控制性卵巢刺激方案* 促性腺激素释放激素拮抗剂(GnRH-ant)和激动剂(GnRH-a)方案是辅助生殖技术中两种主要的控制性卵巢刺激(controled ovarian stimulation, COS)方案。GnRH拮抗剂方案鲜胚移植周期的种植率和临床妊娠率低于GnRH激动剂方案, 但两种方案的累积活产率无显著差异[80]。小鼠实验结果显示, GnRH拮抗剂方案子宫内膜容受性标志物如HOXA10、LIF和αvβ3等因子的表达水平降低, 因此认为拮抗剂方案鲜胚移植周期子宫内膜容受性受损是其主要原因[80-81]。

CHEN等[82]对两种方案患者分泌中期子宫内膜组织行蛋白质组学分析, 发现362个蛋白显著差异表达, 其中87个蛋白与能量代谢、维持细胞骨架稳定性相关。XU等[83]研究发现, 一种与炎症和同种异体排斥反应相关的细胞因子同种异体移植物炎症因子-1(allograft inflammatory factor-1, AIF-1)在GnRH拮抗剂方案内膜中表达水平增加, 并介导着床期TNF-α表达水平增加, 可能损害了子宫内膜容受性。

另外一项体外研究比较了GnRH拮抗剂方案和自然周期患者的分泌中期子宫内膜组织, 拮抗剂组子宫内膜B型肌酸激酶(B-type creatine kinase, CKB)和HOXA10的表达水平显著降低, 推测可能导致肌动蛋白的聚合受阻、细胞骨架损伤和迁移失败[84]。XU等[85]研究认为GnRH拮抗剂通过降低c-kit受体的表达致子宫内膜基质细胞生长不良从而降低子宫内膜容受性。ZHANG等[86]研究发现, 在拮抗剂组患者的子宫内膜容受性标志物S100P表达显著降低、抗凋亡标志物Bcl-2表达降低, 致子宫内膜细胞凋亡增加, 提示S100P可能作为GnRH拮抗剂方案改善IVF妊娠结局的潜在临床靶点。

2.4.2 *冻融胚胎移植周期内膜准备方案* FET因具有预防卵巢过度刺激综合征、可进行胚胎植入前遗传学检测等优点, 在辅助生殖人群中得到广泛应用。自然周期(natural cycle, NC)和激素替代治疗(hormone replacement treatment, HRT)周期、促排卵周期是目前常用的FET内膜准备方案, 三种方案在活产率和孕产结局方面的优劣目前尚无定论。

LOU等[87]发现具有正常排卵的不孕妇女行FET, 与HRT周期(OR 1.18, 95% CI 1.06~1.33)和NC周期(OR 1.24, 95% CI 1.11~1.41)相比, 来曲唑促排卵组具有更高的活产率、临床妊娠率和足月分娩率。对于PCOS等无排卵的不孕症女性, 国际循证指南建议来曲唑为促排卵的一线用药方案[88]。一项在PCOS患者FET周期使用促排卵方案和HRT方案的研究发现, 两组的临床妊娠率、活产率、妊娠丢失率的差异均无统计学意义[89]。而在WANG等[90]的回顾性队列研究中得出相反的结论, 与HRT组相比, 促排方案组有更高的临床妊娠率、活产率, 而流产率、妊娠期高血压疾病(hypertensive disorders of pregnancy, HDP)、妊娠期糖尿病的风险降低, 其他产科结局如早产、剖宫产、小于胎龄儿或大于胎龄儿在两种子宫内膜准备方案中无显著差异。

GnRH激动剂联合激素替代治疗(GnRHa-HRT)作为一种特殊FET方案, 已被证明在子宫腺肌症、反复种植失败和薄型子宫内膜患者中取得了良好的助孕结局[91-94]。目前的文献提示, 对于PCOS患者和高龄女性, GnRHa-HRT方案并不能显著提高活产率[95-96]。

基于GnRH-a鲜胚移植方案具有更高的子宫内容受性、鲜胚种植率和临床妊娠率[80], 降调节诱导排卵(down-regulation ovulation-induction, DROI)方案也用于FET前内膜准备, 旨在改善FET的移植结局。LI等[97]对比了DROI方案和NC方案在FET周期

中临床移植结局发现, DROI组的种植率、临床妊娠率和持续妊娠率均显著高于NC组。一项针对289名单囊胚移植的患者的研究显示, DROI组临床妊娠率和持续妊娠率显著高于HRT组; 两组早期妊娠丢失情况比较差异无统计学意义[98]。

对于GnRH-a降调节与子宫内膜容受性增加的可能机制, 已有研究提示应用长效GnRH-a方案后子宫内膜容受性的生物标志物*HOXA10*、*MEIS1*和*LIF*基因的表达上调[80], 整合素αvβ3的表达能力增强[99], miR-124-3p调控的子宫内膜基质细胞IL-6和IL-11的表达能力增强[100], 这些基础研究为临床检测和治疗提供了新的思路。

2.4.3 子宫内膜致密化 子宫内膜致密化是指胚胎移植日子宫内膜厚度较孕激素转化之前的子宫内膜厚度更薄的现象, 目前认为其可能和超生理剂量的雌孕激素比例有关[101]。2019年BU等[102]研究中曾发现子宫内膜扩张型患者的妊娠率较高。但2020年至今的多项研究提示了相反的结果。两项回顾性队列研究在HRT-FET周期中经腹超声测量子宫内膜厚度变化, 分析发现子宫内膜内膜致密化与持续妊娠率之间存在显著的正相关性[101]。ZIBERBERG等[103]在HRT周期移植PGT-A整倍体单囊胚的观察性队列研究中也发现, 内膜厚度压缩组的妊娠率较无变化组升高, 且子宫内膜致密化程度与活产率之间存在显著相关性。

最近尚有一项进行中的多中心前瞻性队列研究, 研究目标为子宫内膜致密化对改良自然周期中行整倍体胚胎FET后持续妊娠率的影响, 共纳入206名无子宫和子宫内膜疾病且无反复种植失败或反复流产史的女性, 根据转化日与胚胎移植日之间子宫内膜厚度下降程度分为暴露组(≥5%)和非暴露组(<5%), 此研究结果可能为自然周期子宫内膜准备过程中子宫内膜致密化现象对人身结局的潜在影响提供有价值的结论[104]。

对子宫内膜致密化与妊娠结局之间的研究需要关注设计上的异质性, 例如研究人群基线数据、子宫内膜准备方案、超声类型以及移植胚胎的数量和发育天数等影响因素。此外, 还需要对内膜致密化的发生机制进行进一步的阐明。更多设计严谨、大样本前瞻性的RCT研究才能为其临床应用提供更可靠的循证依据。

2.4.4 子宫内膜种植窗移位 目前认为子宫内膜容受性不足或WOI移位可能导致胚胎–子宫内膜同步性丧失而导致胚胎植入失败[105], 子宫内膜组织种植窗检测可能通过调整胚胎移植时间进而改善不明原因反复种植失败患者的妊娠结局[106-107]。子宫内膜容受性阵列(endometrial receptivity array, ERA)通过分析与子宫内膜容受性相关的238个差异基因的表达, 有助于识别胚胎着床的WOI, 以确定胚胎移植时机, 指导个体化胚胎移植。ERA比组织学方法更准确, 并且在第一次检测后29~40个月的同一患者中显示结果具有可重复性[108]。一项研究报道ERA指导的pET提高了WOI移位的RIF患者的种植率和持续妊娠率[109]。但是在COZZOLINO等[110]的研究中却发现在自体周期或供者周期中使用ERA并不能改善助孕结局, 应用ERA反而降低了临床妊娠率。一项Mate分析表明, 在FET前应用ERA指导个体胚胎移植时间并不能提高ERA组的活产率或持续妊娠率, 根据既往ET失败次数进行的亚组分析也未见显著差异[111]。KURODA等[112]通过对CE患者行ERA检测发现, CE组子宫内膜容受性显著降低, 对CE组和CE治愈组均进行ERA检测后行FET, CE治愈组的临床妊娠率高于CE组。

3 子宫内膜与辅助生殖人群妊娠并发症及子代健康

大量研究表明, 与自然受孕相比, 辅助生殖助孕母亲子痫前期、妊娠期糖尿病和胎盘异常等妊娠并发症的风险增加[113-114]。其机制复杂且尚不明确, 子宫内膜异常可能是其中重要病因之一。

3.1 hCG日子宫内膜厚度

辅助生殖技术助孕的薄型子宫内膜患者胎盘相关不良妊娠结局发生率较高[115]。薄型子宫内膜患者妊娠早期子宫胎盘螺旋动脉重塑异常, 可导致随后胎盘深层化形成缺陷, 并最终导致子宫胎盘血流减少。研究发现, hCG日子宫内膜厚度<7.5 mm女性单胎妊娠后, 子痫前期、胎盘早剥和宫内生长受限、早产等胎盘相关不良妊娠结局发生率增加[116]。对辅助生殖子代健康而言, hCG日子宫内膜厚度≤7.5 mm组发生小于胎龄儿的风险是子宫内膜厚度>12 mm组的2倍[117]。另一项研究发现, hCG日子宫内膜厚度≤8 mmHDP发生率显著高于子宫内膜厚度>8 mm组[118]。最近一项纳入19个研究, 共76 404个周期的系统综述分析, hCG日子宫内膜厚度≤8 mm

组子代具有较高的胎盘早剥、HDP、剖宫产率、早产、低出生体重和小于胎龄儿风险[119]。

因此, hCG日子宫内膜厚度不仅与胚胎成功着床有关, 也与胎盘和胎儿进一步健康发育密切相关。

3.2 分泌早期子宫内膜致密化

孕激素作用后子宫内膜向分泌期转化, 分泌早期出现的子宫内膜厚度变化与辅助生殖助孕女性和子代健康的影响是值得关注的新的切入点。

2023年GILL等[120]的一项回顾性队列研究对比hCG日/孕激素转化前与移植日内膜厚度, 提示48%患者出现子宫内膜致密化(内膜厚度压缩≥10%), 该组早产率以及胎盘介导的子痫前期、胎盘早剥、死胎和宫内生长受限等并发症的发生率均高于非压实组(内膜厚度压缩<10%), 但受限于样本量较小, 差异无统计学意义。

能否将子宫内膜致密化用于预测冻融胚胎移植后的妊娠结局尚存争议, 目前一项针对自然周期PGT正常信号单囊胚移植的前瞻性队列研究正在进行中, 期待其对母儿远期随访结果能提供更多的循证医学证据。

3.3 冻融胚胎移植周期不同内膜准备方案

近来的研究表明, 黄体不仅分泌雌二醇和孕酮, 还分泌松弛素、催产素、肾素、醛固酮、血管内皮生长因子和其他血管活性化合物[121-122], HRT内膜准备方案因为黄体的缺失可能会增加HDP的发生风险[123-124]。

HU等[125]发现, 与NC周期相比, HRT方案的早产、剖宫产、巨大儿、胎膜早破和HDP的风险增加, 促排卵周期与过期妊娠和妊娠期糖尿病的风险增加有关, 三种子宫内膜准备方法中小于胎龄儿、前置胎盘、先天发育异常差异无统计学意义。另外两项回顾性研究发现HRT方案子痫前期、产后出血和剖宫产的发生率较高[126-127]。LI等[128]的回顾性研究结果提示, HRT方案增加了HDP和妊娠期肝内胆汁淤积症(intrahepatic cholestasis of pregnancy, ICP)的风险, 也增加了FET后单胎和多胎妊娠发生低出生体重和小于胎龄儿的风险。另一项回顾性队列研究也显示, HRT周期与自然周期及促排卵周期相比, HDP的发生风险更高[129]。

4 小结与展望

作为孕育新生命的土壤, 子宫腔曾被视为一个神秘的“黑匣子”, 对子宫内膜的了解甚至一度落后于对配子和胚胎的研究, 但得益于包括单细胞组学、空间转录组学、微生物组学、代谢组学及类器官等强大的基础研究新技术的出现, 我们对子宫内膜在周期性生理修复中非凡的可塑性了解越来越多, 进而推进了我们对胚胎着床及持续妊娠过程中内膜与胚胎间交互作用的研究。辅助生殖技术所服务人群的某些特殊疾病状态, 以及所特有的控制性卵巢刺激药物的使用, 又使得我们必须不断加深对子宫内膜损伤、炎症、应激、衰老和再生等机制的研究, 了解子宫内膜对辅助生殖妊娠结局、母儿孕产结局甚至子代健康的影响, 为进一步提高辅助生殖技术的有效性和安全性提供新角度和新方法。

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