注重妊娠结局的改善研究专题·综述

宫腔灌注改善辅助生殖助孕患者妊娠结局的研究进展

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【关键词】宫腔灌注;IVF-ET;妊娠结局;子宫内膜容受性
【中图分类号】R 711.59;R 711.6 【文献标志码】A 【文章编号】1674-4020(2021)10-009-04
doi:10.3969/j.issn.1674-4020.2021.10.03

目前,随着辅助生殖技术的发展,体外受精-胚胎移植(in vitro fertilization-embryo transfer,IVF-ET)患者的妊娠结局不断得到改善,然而仍有部分患者出现胚胎反复种植失败。在导致胚胎种植失败的诸多因素中,内膜因素是影响胚胎着床的重要因素之一[1]。为提高妊娠率,在胚胎移植前有多种经验性方法提高子宫内膜的容受性,除药物治疗(如阿司匹林等)、子宫内膜搔刮(和/或联合宫腔镜检查)外[2-5],宫腔灌注是近年来出现的一种新的治疗方法,是向宫腔内灌注某些药物或因子以达到刺激子宫内膜,改善子宫内膜容受性的一种方法。通常在胚胎移植前 1~3 天进行[6]。其灌注的物质包括人绒毛膜促性腺激素(human chorionic gonadotrophin,hCG)、粒细胞集落刺激因子(granulocyte colony-stimulating factor,G-CSF)、外周血单个核细胞(peripheral blood mononuclear cells,PBMC)、地塞米松、低分子肝素、富血小板血浆(platelet-rich plasma,PRP)等。不同的灌注物,其作用机理和优缺点各不相同。依据灌注物种类不同,本文就宫腔灌注对改善辅助生殖助孕患者妊娠结局的研究进展进行综述。

1 人绒毛膜促性腺激素

1.1 作用机理与方法

Mansour 等[7]首次报道了宫腔灌注 hCG 可显著提高胚胎种植率和临床妊娠率。其可能作用机理如下:首先,hCG 在自然受孕和辅助生殖中起着非常重要的作用,它可维持黄体中黄体酮的产生,用于黄体期支持,同时,hCG 可激活子宫肌层细胞中 Ca^{2+} 激活的 K^+ 通道(BK 通道)从而抑制子宫肌层收缩,安静子宫平滑肌从而有利于胚胎的种植[8]。其次,Paiva P 等[9]指出 hCG 可显著促进子宫内膜上皮细胞(human endometrial epithelial cells,hEEC)中因子的产生,包括已知与容受性和滋养层功能有关的因子[白细胞介素(interleukin,IL)-11]、囊胚迁移和粘附因子(CXCL10)、囊胚发育因子(G-CSF)和与容受性和种植有关的未知因子[成纤维细胞生长因子 2(fibroblast growth factor 2,FGF 2)],从而对子宫内膜的容受性及胚胎的种植起着重要的作用。第三,在胚胎移植过程中 hCG 能调节子宫内膜基质细胞的容受性、促进内皮细胞增殖及调节 IL-1 受体[10],其中 IL-1 是胚胎最早释放的信号之一,是对容受性子宫内膜并诱导胚胎植入必不可少的主要分子,hCG 可靶向内皮细胞,并调节内皮细胞对 IL-1 的反应,从而直接促进子宫内膜的血管生成并促进胚胎的生长发育[11]。

上述作用机理可能在提高胚胎种植率方面发挥了重要作用。在新鲜周期或冷冻周期中,常选择 500 IU~1 000 IU hCG 灌注至宫腔内,继而将胚胎移植至宫腔内[12]。

1.2 对妊娠结局的影响

目前对于宫腔灌注 hCG 是否能改善妊娠结局并无一致的结论。有 Meta 分析研究提出宫腔灌注 hCG 能显著提高患者的临床妊娠率和持续妊娠率[13],尤其对于两次及以上胚胎移植失败的患者,宫腔灌注 hCG 能显著改善胚胎种植率和临床妊娠率[14-16]。

相反,也有 Meta 分析研究指出宫腔灌注 hCG 对于活产率及流产率并无改善作用[12],Kathleen HH [17]和 Hafezi M[18]报道在囊胚移植期(新鲜或冷冻周期)宫腔灌注 hCG 并不会提高胚胎种植率和临床妊娠率。移植前灌注 hCG 使子宫内膜长期暴露于 hCG,反而导致子宫内膜失去了对胚泡分泌的 hCG 的反应[12,19]。此外,灌

注至宫腔的 hCG 与胚泡分泌的 hCG 生理学时项不同步,这可能也是导致胚胎移植失败的原因之一。此外,不同型别的 hCG(如高糖基化的 hCG、β-hCG)由胚胎、细胞滋养层和合胞体滋养层依据胚胎的发育阶段以不同的优势水平产生[20],通过灌注高剂量的 hCG(尿源性或重组)可能无法实现对不同亚基 hCG 的精准调控,且 hCG 的有效生理功能或许仅能由发育正常的胚胎产生。

2 粒细胞集落刺激因子

2.1 作用机理与方法

在生殖系统中,G-CSF 由颗粒细胞产生,有助于卵泡发育、排卵和卵巢反应[21-22],其通过上调滋养层细胞中金属蛋白酶-2 促进胚胎的植入并参与滋养层功能的调节[23]。其次,G-CSF 主要促进子宫内膜树突状细胞的聚集、Th-2 细胞因子的分泌和 T 调节细胞的激活,并影响子宫内膜中关键基因的表达,这对于局部免疫调节、子宫内膜血管重塑和细胞粘附至关重要[24]。此外,G-CSF 可刺激中性粒细胞增殖和分化,并作用于蜕膜细胞的巨噬细胞,从而影响植入[25]。

薄型子宫内膜通常定义为 hCG 日内膜厚度 < 7 mm[26],有研究表明子宫内膜厚度 <7 mm 与临床妊娠率较低有关[27]。既往可采用延长雌激素给药时间、使用血管活性药物(如低剂量阿司匹林、枸橼酸西地那非、己酮可可碱-生育酚)和干细胞治疗薄型子宫内膜。然而,仍有少部分患者的子宫内膜对上述治疗无反应,从而导致取消移植周期或移植失败。近年来,宫腔灌注 G-CSF 常用于治疗薄型子宫内膜患者[28-29]。然而,目前对于 G-CSF 是否是依靠增加子宫内膜的厚度从而改善妊娠结局仍不明确[30-31]。在取卵日或胚胎移植日,常选择 300 ug/mL的 G-CSF 灌注至宫腔中,继而在新鲜周期或冷冻周期中将胚胎移植至宫腔内[32]。

2.2 对妊娠结局的影响

宫腔灌注 G-CSF 可能改善薄型子宫内膜患者的妊娠结局,而对正常厚度的子宫内膜患者无效。在 Xie YR[30] 的研究中,相对于对照组,宫腔灌注组增加了子宫内膜厚度、临床妊娠率、胚胎移植率,同时也降低了周期取消率。然而也有研究表明,灌注 G-CSF 并没有增加子宫内膜的厚度,这也许是因为 G-CSF 可作为一种免疫疗法改善患者的妊娠结局[31-32]。此外,Barad DH 等[32] 指出 G-CSF 并不能改善子宫内膜厚度、胚胎种植率和临床妊娠率,但由于其研究人群年龄为(39.59 ± 5.56)岁,其也提出宫腔灌注 G-CSF 可能对年轻女性更有效的设想。Eftekhar M 等[32-33] 则提出 G-CSF 对于正常厚度的子宫内膜人群无效。

3 外周血单个核细胞

3.1 作用机理与方法

近年来,越来越多研究表明子宫内膜局部免疫细胞对胚胎的着床起到了重要作用,胚胎的着床是内分泌系统和免疫系统共同作用的结果[34]。Fujiwara H[35-36] 提出 hCG 可刺激黄体细胞生成黄体酮,从而维持胚胎在宫腔内的植入,研究表明,PBMC 同样也可刺激黄体细胞生成黄体酮,并且其能激活 cAMP 诱导 Treg 细胞的迁移和活化及分泌 TGF-β 和 IL-10 等细胞因子调节母胎界面的免疫耐受从而促进并维持妊娠进展。此外,PBMC 诱导 IL-1α、IL-1β 和 TNF-α 细胞因子的产生,这些因子对子宫内膜以及子宫内膜容受性具有积极作用[35,37]。从患者体内分离出自体 PBMC,在新鲜周期或冷冻周期胚胎移植前,将新鲜分离的 PBMC 细胞(1 ~ 3 × 10^7 细胞)注入患者宫腔中[38-41]。

此外,也有研究采用宫腔灌注 hCG 孵育后的 PBMC。Fujiwara 提出了通过 hCG 孵育的活化的 PBMC 促进胚胎植入三种可能机制。首先,宫腔灌注活化的 PBMC 可以诱导胚胎植入的子宫内膜分化,继而促进胚胎种植。其次,PBMC 可以在宫腔中引起有利的炎症反应,分泌的蛋白酶可以有效改变在宫腔内膜上皮细胞上表达的表面分子的功能或结构。第三,在宫腔灌注后,PBMC 可以从宫腔向子宫内膜基质组织移动,形成一个为后续胚胎附着和侵袭的主要途径[42]。

3.2 对妊娠结局的影响

对于宫腔局部灌注 PBMC 对妊娠结局的影响,有研究指出宫腔灌注 PBMC 对多次胚胎种植失败的患者妊娠结局明显优于对照组[38,43-44]。Li SJ[39] 认为宫腔灌注 PBMC 尤其能改善 4 次及以上种植失败的患者的妊娠结局,而 1 ~ 3 次种植失败病史的患者并不能从中获益。

4 其他

4.1 地塞米松

Zhang T[40] 指出子宫自然杀伤细胞(uterine natural killer cell,uNK)与反复胚胎种植失败有关,研究表明:在反复种植失败并宫腔内高 uNK 患者宫腔灌注地塞米松药物,能够下调 uNK 的比例,从而改善子宫内膜的容受性并一定程度改善妊娠结局。然而,由于该研究样本量偏小,且为非随机对照研究,外加关于地塞米松在宫腔中的代谢机制仍不明确,因此宫腔灌注地塞米松的安全性及有效性仍需进一步研究。

4.2 低分子肝素

近期的一项 Cochrane 评价显示,目前尚不清楚全身使用肝素在 IVF 周期中是否能改善不孕女性的妊娠率和活产率,然而他们提出辅助生殖技术中在子宫局部应用肝素的设想[41]。在 Kamel AM 等[45] 的研究中,将卵胞浆内单精子注射(intracytoplasmic sperm injection,ICSI)女性在取卵术后胚胎移植前 2 ~ 5 天宫腔灌注 500 IU 低分子肝素,最终得出研究组与对照组临床妊娠率和胚胎种植率并无差异。

4.3 自体富血小板血浆

PRP 目前广泛应用于骨科、眼科等以促进伤口愈合,关于其宫腔灌注能够促进子宫内膜生长并改善薄型子宫内膜患者的妊娠结局最早是由 Chang 等[46] 提出的,用自体血离心制备 PRP,在激素替代疗法周期第 10 天

宫腔内注入 0.5 ~ 1 mL，若 72 h 后子宫内膜厚度仍未增加，则每周期输注 PRP 1 ~ 2 次，当子宫内膜厚度 >7 mm 时移植胚胎。通过激活 PRP 中的血小板，细胞因子和生长因子变得具有生物活性，这些因子包括血管内皮生长因子、转化生长因子、血小板衍生生长因子和表皮生长因子，它们可以调节细胞的迁移、粘附、增殖和分化，促进细胞外基质的积累[47]。

5 宫腔灌注应用的安全性与展望

宫腔灌注通过局部给药，可使局部达到更高的药物浓度、全身不良作用小及成本更低，然而药物在宫腔中的代谢情况仍不明确，灌注的药物在宫腔上皮的作用位点及其是否会引起宫腔积液从而影响胚胎种植仍未可知，此外，宫腔操作增加了上行感染的风险。目前，由于各项宫腔灌注研究纳入的样本量小，仍需要大量的随机对照研究提供可靠的循证医学证据证实其临床有效性。

所有作者均声明不存在利益冲突

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(收稿日期:2020-04-30 编辑:杨叶)