

牙周炎促进不良妊娠结局的途径与作用机制*

关志伟^{1,2}, 徐天琪¹, 沈松¹, 李晓³, 冯强^{1△}

1. 山东大学齐鲁医学院口腔医学院·口腔医院 人体微生态实验室 山东省口腔组织再生重点实验室

山东省口腔生物材料与组织再生工程实验室 山东省口腔疾病临床医学研究中心(济南 250012);

2. 齐鲁师范学院生命科学学院(济南 250200); 3. 山东大学生殖医学研究中心 生殖内分泌教育部重点实验室(济南 250012)

【摘要】 牙周炎是人群中高发的口腔慢性感染性疾病,牙周致病菌可通过血液传播途径异位定植并感染多个人体组织器官,是多种全身系统性疾病的危险因素。近年来,牙周炎与不良妊娠结局(adverse pregnancy outcomes, APOs)间的关系引起越来越多的关注。本文系统回顾牙周炎与APOs相互关系的研究历程,总结已有牙周炎促进APOs的致病途径和机制,阐明口腔致病菌经血液传播造成宫内感染是牙周炎干扰妊娠过程的重要途径。未来如要深入解析牙周炎影响APOs的具体分子机制,可将研究重点放在APOs相关口腔致病菌及其毒力因子的发现、致病菌与胎盘组织的相互作用分析及侵袭胎儿的致病途径等方向。此外,通过动物和细胞实验验证人群研究的结果并将其转化为行之有效的干预措施对防控APOs的发生、发展具有重要的临床意义。

【关键词】 牙周炎 不良妊娠结局 牙周致病菌 宫内感染 具核梭杆菌

Pathways and Mechanisms of Periodontitis Contributing to Adverse Pregnancy Outcomes GUAN Zhi-wei^{1,2}, XU Tian-qi¹, SHEN Song¹, LI Xiao³, FENG Qiang^{1△}. 1. Department of Human Microbiome, School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University, Shandong Key Laboratory of Oral Tissue Regeneration, Shandong Engineering Laboratory for Dental Materials and Oral Tissue Regeneration, and Shandong Provincial Clinical Research Center for Oral Diseases, Jinan 250012, China; 2. School of Life Science, Qilu Normal University, Jinan 250200, China; 3. Center for Reproductive Medicine, Shandong University and Key Laboratory of Reproductive Endocrinology of Ministry of Education, Jinan 250012, China

△ Corresponding author, E-mail: fengqiang@sdu.edu.cn

【Abstract】 Periodontitis is a chronic oral inflammatory disease with a high incidence in the global population. Periodontal pathogens can colonize and infect multiple human tissues and organs through blood transmission, which is an important risk factor of many systemic diseases. Recently, the correlation between periodontitis and adverse pregnancy outcomes (APOs) has attracted growing research interest. Herein, we systematically reviewed the research progress in the relationship between periodontitis and APOs and summarized reported findings on the pathways and mechanisms by which periodontitis contributes to APOs. We also clarified that intrauterine infection caused by oral pathogens transmitted through blood is an important pathway by which periodontitis interferes with pregnancy. In addition, further research focused on the discovery of more APOs-related oral pathogenic bacteria and their virulence factors, analysis of the interaction between pathogenic bacteria and placental tissue, and pathogenic pathways of oral bacterial invasion of the fetus will promote thorough analysis of the specific molecular mechanism of how periodontitis affects APOs. Furthermore, the validation of the results of human population-based studies through animal/cell experiments and the translation into effective intervention strategies are of great clinical significance to the prevention and control of the occurrence and development of APOs.

【Key words】 Periodontitis Adverse pregnancy outcome Periodontal pathogens Intrauterine infection *Fusobacterium nucleatum*

近年来,口腔健康与全身健康的关系引起越来越多的关注和重视,牙周致病菌在促进全身炎症及导致特定器官功能障碍中的作用已被广泛证实,如牙周致病菌可引发或促进心血管疾病、卒中、糖尿病、肺炎、慢性肾

病、脑膜炎、类风湿性关节炎、阿尔茨海默病、多种肿瘤及不良妊娠结局(adverse pregnancy outcomes, APOs)等^[1-2]。

APOs包括流产、早产、先兆子痫及子痫、死胎、死产、低出生体重儿等。1996年OFFENBACHER等^[3]率先报告患有牙周炎的女性早产及新生儿低出生体重风险较对照组增加了近7倍,随后的大量研究结果证实牙周炎对APOs具有不利影响。然而,牙周炎虽已被广泛认可可是APOs的独立和潜在的危险因素,但其具体致病机制尚不

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△ 通信作者, E-mail: fengqiang@sdu.edu.cn

完全清楚。

本篇综述通过总结、梳理牙周炎促进APOs的可能途径及相关致病机制的研究证据,分析了当前牙周炎影响APOs的研究所面临的挑战,并对未来的研究方向进行了展望。

1 妊娠对口腔健康状况变化的影响

妊娠期的生理变化会增加口腔疾病的易感性。首先,妊娠早期早孕反应造成的呕吐或妊娠晚期的胃反流会导致胃酸进入口腔并腐蚀牙齿^[4];其次,妊娠期性激素水平会发生显著升高,这些激素的受体在各种牙龈组织/细胞中普遍存在,可引发牙周组织的炎症反应,这些性激素在妊娠期的特异性升高可能导致孕妇牙周炎症的激发或加重^[5],且孕前已患牙龈炎女性的症状在妊娠期间会随着妊娠的进展而加重^[6];此外,妊娠相关雌激素和孕酮水平的显著升高及妊娠期免疫反应减弱、饮食习惯等发生的变化(如精制糖和蛋白质的增加)可促进牙菌斑相关致病微生物的生长、增加牙龈组织中血管的通透性,是牙周疾病在孕妇中发病率升高的重要诱因^[7]。

同时,牙周病增加APOs的发病风险。研究显示,牙龈炎在孕妇中的发病率高达60%~75%^[8]。进行性牙龈炎可发展为牙周炎,该病以牙周组织的破坏和牙槽骨丢失为主要症状,是导致成年人牙齿脱落的主要原因^[9]。口腔生态研究揭示牙周炎的起因在于“关键病原体”以损害免疫监测的方式调节宿主反应,进而将口腔生态的稳态平衡转变为“多种致病微生物协同作用”与免疫功能紊乱的生态失调状态^[10]。SOCRANSKY等^[11]将牙周炎龈下菌斑中的细菌划分为不同的微生物复合体,随着牙周炎严重程度增加,具有明显致病性的“橙色”细菌复合体[包括具核梭杆菌(*Fusobacterium nucleatum*, Fn)、直肠弯曲杆菌(*Campylobacter rectus*, Cr)、*Peptostreptococcus micros*、*Prevotella intermedia*以及*Prevotella nigrescens*等]出现,并为更具致病性的“红色”复合体[包括牙龈卟啉单胞菌(*Porphyromonas gingivalis*, Pg)、*Tannerella forsythia*和*Treponema denticola*等]的后续定殖和组织侵袭提供必要的栖息环境。研究显示,龈下菌斑中Pg和中间普氏菌(*P. intermedia*)的丰度分别与孕期母体激素水平及牙周组织破坏程度呈正相关^[12-13]。牙周炎的进展伴随着许多牙周致病菌的定殖、增殖并侵袭牙周组织,刺激牙周组织/细胞分泌促炎因子和炎症介质、招募大量炎症反应相关免疫细胞,导致牙周血管大面积破损;大量炎症介质和致病菌由此侵入血管经血液循环到达远端组织并定殖,引发远端组织炎症,进而促进多种全身系统性疾病的发生发展^[14]。在现有报道中,不同地区孕妇牙周炎的患病率差

异较大,可能是因为不同地区口腔卫生干预状况和牙周炎诊断方式及诊断标准的不同^[15]。

上述研究报道说明,妊娠期间与口腔健康状况改变之间的影响是相互的。妊娠期生理特征、激素水平、孕妇饮食结构及口腔卫生干预的减少导致口腔微生态组成的变化,增加口腔疾病的发病风险,而以牙周炎为代表的口腔疾病则会增加APOs的发病风险。

2 炎症是促进APOs的重要病因之一

妊娠是非常复杂且变化极为迅速的生理过程,胚胎植入母体是妊娠的开始。胚胎植入后,滋养层细胞进一步侵入子宫内膜发育为胎盘,胎盘血管丰富,母亲和胎儿之间通过胎盘进行营养和代谢产物交换,保证胎儿生长必需的营养供给和代谢产物排出。女性性激素水平在妊娠期间会发生明显波动,血浆孕酮和雌激素在孕晚期达到峰值水平,分别是月经来潮期间的10倍和30倍^[16]。这些激素,尤其是孕酮,在母亲的免疫反应过程中不仅保护母亲及胎儿免受外部病原的侵害,且对孕妇耐受胎儿也至关重要,因为胎儿携带了外源的父方DNA,具有同种异体移植物的效应,为了防止胎儿免受免疫排斥、维持妊娠,外周血和母胎界面都发生了从辅助性T细胞(T helper, Th)1和Th17向Th2和调节性T细胞(T regulatory, Treg)的免疫应答转变^[17-18]。随着妊娠的进展,胎儿不断生长,发育到成熟阶段并最终分娩。分娩启动的原因尚无定论,现认为分娩启动是多因素综合作用的结果,其中最主要是孕酮的下降和催产素的上升导致的神经-生殖轴的启动^[19]。此外,炎症反应的调控也是促进分娩的重要原因,分娩前羊水中前列腺素E2(prostaglandin E2, PGE2)和肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)、白细胞介素-1 β (interleukin-1 β , IL-1 β)等炎症细胞因子的浓度逐渐上升,当达到临界阈值时,子宫开始剧烈收缩,导致宫颈扩张、羊膜囊破裂和最终的分娩^[20]。

多种外部因素会引发APOs,例如生存环境、生活方式、营养条件、社会经济因素、遗传背景和外源微生物干扰等^[21],而局部或全身炎症介质升高是外部因素造成APOs的重要内因。首先,人群研究发现羊水或母体血清中IL-1 β 、IL-6、IL-8或TNF- α 水平的升高与早产有关^[22-24];宫内TNF- α 和IL-1 β 等细胞因子水平的增加激活基质金属蛋白酶,该酶能够降解羊膜囊膜的胞外基质,从而诱导羊膜囊破裂造成流产或早产^[25]。其次,肝脏响应病原菌感染或细胞因子刺激产生炎症标志物C反应蛋白(C-reactive protein, CRP),其在血浆中的升高被证实与羊膜炎和早产有关^[26-27];除早产外,CRP水平升高也会增加先

兆子痫和宫内生长受限等其他APOs的风险^[28]。再次,在先兆子痫孕妇的胎盘和脐带血中分别检测到免疫细胞组成的异常变化,具体表现为Th2、Treg活性地降低与Th1、Th17活性地升高^[29-30],血管生成因子和抗血管生成因子之间的不平衡也被认为是先兆子痫的潜在病因^[31]。血清CRP、IL-6和TNF- α 的升高会诱发妊娠期糖尿病(gestational diabetes mellitus, GDM),因为IL-6和TNF- α 是胰岛素拮抗剂,其持续升高会干扰糖代谢,从而导致糖耐量受损,造成GDM^[32]。此外,源于生殖器官(如阴道、宫颈和输卵管)或非生殖器官的微生物能侵入子宫(如通过血行传播或侵入性羊膜穿刺术意外引入等途径),可在绒毛膜腔、绒毛膜、羊水、胎盘、脐带和胎儿等部位引起感染,宫内感染造成的损伤以及过度的炎症反应可通过早期羊膜囊破裂和子宫收缩导致流产或早产^[33-34]。

3 牙周炎促进APOs的途径与作用机制

如前所述,牙周炎是一种发生在距离胎盘-胎儿较远部位的感染性疾病。为了消除或抑制牙周感染,宿主牙周组织/细胞激活针对感染的细菌及其毒力因子的局部

炎症反应,而炎症因子、牙周致病菌或其毒力因子可趁机进入血液循环并在全身传播,触发全身炎症反应或诱导异位感染。通过总结该领域的系列研究进展,BOBETIS等^[35-36]在2006年提出并于2020年完善了牙周炎影响APOs的致病机制模型。该模型认为牙周炎可通过多种方式促进APOs,如牙周致病菌及其致病组分传播到胎盘-胎儿,引发异位感染和组织损伤,或触发感染部位炎症反应,导致炎症因子和炎性介质升高;牙周致病菌刺激牙周组织产生炎症因子和炎性介质,通过血液循环到达胎盘-胎儿并在其中大量积累,引发宫内炎症;血液中的病原菌、细胞因子和炎性介质进入并刺激肝脏产生急性期反应物(如CRP)激活全身炎症反应,产生的炎症介质通过血液循环进入胎盘-胎儿,加剧宫内炎症等。

笔者基于该领域的研究进展和本团队在牙周致病菌致病机制的认识和理解,在BOBETIS等报道的基础上系统梳理了相关的研究资料和文献,总结了牙周炎促进APOs的三种主要途径(图1)。

3.1 牙周致病菌经口至子宫异位定植

目前牙周炎促进APOs的较为明确的原因之一是牙

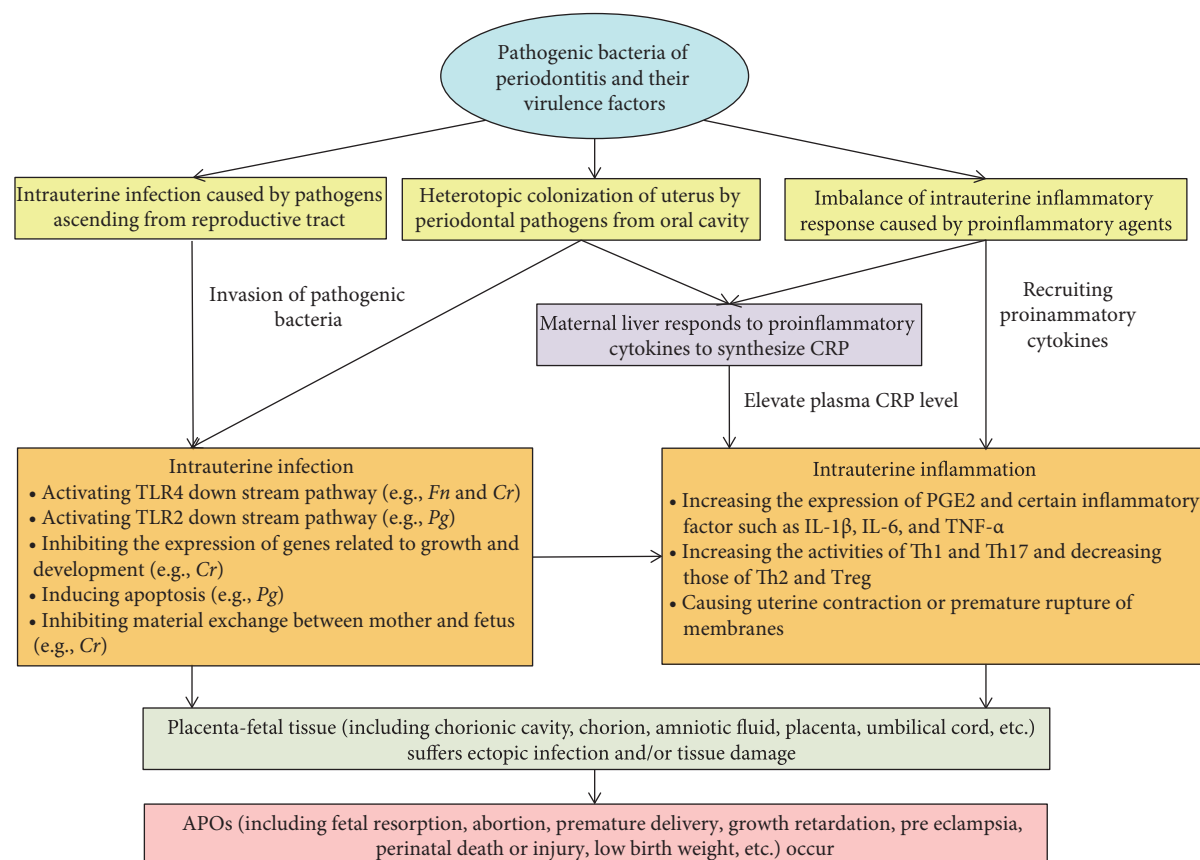


图1 牙周炎促进APOs的致病途径与作用机制

Fig 1 Pathogenic pathway and mechanism of periodontitis promoting APOs

Fn: *Fusobacterium nucleatum*; *Cr*: *Campylobacter rectus*; *Pg*: *Porphyromonas gingivalis*; CRP: C-reactive protein; TLR: toll-like receptor; PGE2: prostaglandin E2; IL: interleukin; TNF: tumor necrosis factor; Th: T helper; Treg: T regulatory; APOs: adverse pregnancy outcomes.

周致病菌经血运途径从口腔异位定植于胎盘并进入羊水,接触并感染胎儿。牙周炎发病过程中,龈沟液中的致病菌与牙周袋内的牙周膜密切接触,引发局部炎症使致病菌及其毒力因子可侵入到牙周组织继而进入牙周血管中,引发菌血症,该过程或可发生在口腔清洁、牙周治疗造成的牙龈出血过程中^[37]。迄今为止,由于多数口腔厌氧菌难以培养,我们对牙周炎通过该途径影响APOs机理的认知主要来源于病例研究、人群队列研究及可培养的牙周炎“关键病原体”(如*Fn*、*Cr*以及*Pg*)在动物及细胞学方面的实验证据,表1为相关的机制研究证据总结。

HAN等发表的两项病例研究为牙周炎致病菌异位定植导致APOs的致病模式提供了临床证据。第一项研究^[38]发现,在一名早产妇女的龈下菌斑和羊水中检测到同一不可培养的*Bergeyella*菌株DNA,但在患者的阴道样本中未检测到该菌株。在第二项研究^[39]中,一名患有牙龈炎的孕妇在妊娠39周时出现上呼吸道感染症状,几天后发生死产,研究人员从其胎盘和胎儿中分离出*Fn*,并在该孕妇的龈下菌斑中发现相同的菌株,但并未在其阴道和直肠样本发现该菌株,这说明在呼吸道感染导致免疫力减弱时,*Fn*可能从孕妇口腔经菌血症转移至子宫。

*Fn*是龈下菌斑中最常见的口腔条件致病菌之一,在正常情况下身体的其他部位很少检出。然而,*Fn*在多种全身系统性疾病的病灶部位被高频检出,且*Fn*抗体的滴度伴随着疾病的进展有所升高^[40]。*Fn*在牙周炎和APOs的病灶部位均被高频检测到,且是为数不多的能够实现体外纯种培养的口腔厌氧菌之一,所以*Fn*是研究牙周炎和APOs关系的“模式菌种”。2004年,HAN等^[41]模拟口源性菌血症的感染途径向受孕的CF-1小鼠静脉注射口腔来

源的*Fn*,导致小鼠早产、死产和非持续活产率显著增加,分析发现从受孕小鼠的胎盘、羊水和胎儿中均可分离出*Fn*,但相同感染时间序列下肝脏和脾脏中的活菌却逐渐被清除,这说明口腔来源*Fn*的异位感染偏好于在宫腔内定植;对感染小鼠的子宫进行免疫组化分析发现*Fn*感染及其造成的炎症集中在胎盘的底蜕膜和静脉窦,透射电镜观察发现*Fn*有从静脉窦内迁移并在底蜕膜中增殖的趋势;免疫组化分析和透射电镜观察的实验结果相互支持,推断静脉窦中缓慢的血流速度和由此产生的低剪切力为*Fn*粘附和侵入内皮细胞及随后感染底蜕膜提供了机会。此外,该研究还指出在妊娠前向小鼠注射*Fn*不太可能导致APOs,说明胎盘是*Fn*定植于生殖系统的前提条件^[41]。这项研究是证明口腔致病菌经血液传播导致APOs最具开创性的实验证据之一。接下来,该团队通过对*Fn*毒力因子的发现与研究进一步揭示了*Fn*造成牙周组织感染及定植特定组织器官的具体分子机制。黏附素蛋白FadA是*Fn*第一个被证实的毒力因子^[42],是*Fn*附着和侵入宿主上皮细胞和内皮细胞常用的黏附蛋白^[43],FadA与血管内皮钙黏蛋白结合,导致紧密连接松动,使*Fn*及其他口腔细菌得以穿透内皮^[44],*fadA*基因缺失的*Fn*菌株在胎盘定植中存在显著缺陷^[45]。定位于*Fn*外膜的半乳糖黏附凝集素Fap2是另一个重要的毒力因子,Fap2介导*Fn*对*Pg*等其他病原菌和多种宿主细胞的附着,细胞表面的半乳糖-N-乙酰-D-半乳糖胺(Gal-GalNAc)是Fap2的特异性结合受体,可促进*Fn*在胎盘定植^[46-47]。值得注意的是,HAN团队发现*Fn*一旦在胎盘中定植,就会扩散到羊水、胎膜和胎儿并诱导toll样受体4(toll-like receptor 4, TLR4)依赖的炎症反应,造成中性粒细胞浸润,从而导致早产或死产;而在

表 1 牙周致病菌从口腔异位定植于子宫相关研究文献总结

Table 1 Summary of research reports on the heterotopic colonization of periodontal pathogens from oral cavity to uterus

Experimental method	Conclusion and importance
Animal experiment ^[41, 50, 57]	Confirming the possibility that periodontal pathogens (e.g. <i>Fn</i> , <i>Gr</i> , and <i>Pg</i>) could cause intrauterine infection via blood circulation, and then lead to APOs
Animal experiment ^[45, 65] Cell experiment and animal experiment ^[46] Tissue microarray analysis and animal experiment ^[47]	Confirming that specific virulence factors (e.g. FadA and Fap2 of <i>Fn</i> and gingipains of <i>Pg</i>) play a key role in the process of APOs caused by periodontal pathogens and these virulence factors are potential targets for clinical intervention
Animal experiment ^[48, 52, 58-60]	Suggesting that the inflammatory imbalance caused by periodontal pathogens via intrauterine infection is an significant factor inducing APOs
Animal experiment ^[51]	Suggesting that the intrauterine infection caused by periodontal pathogens via blood circulation hampers the material exchange between the mother and the fetus. The damage affects not only pregnancy, but also offspring growth and development in the perinatal period
Animal experiment ^[53-54]	Indicating that periodontal pathogens can cause APOs by inhibiting the expression of specific genes (e.g. at the epigenetic level)
Cell experiment ^[61-63]	Suggesting that periodontal pathogens can promote APOs by inducing apoptosis

The abbreviations are explained in the footnote to Fig 1.

TLR4敲除小鼠或经TLR4拮抗剂治疗的野生型小鼠中,*Fn*在胎盘上的定植程度与未经治疗的野生型小鼠相似,但死产率却显著减少,这说明导致小鼠死产的原因是*Fn*引起的炎症反应,而不是由*Fn*感染直接引起^[48]。

*Cr*是一种革兰阴性的牙周致病菌,可以在微需氧或厌氧状态下培养。*Cr*和*Fn*同为“橙色”复合体成员,*Cr*感染与APOs之间亦关系密切。2001年,MADIANOS等^[49]研究发现早产儿脐带血清中*Cr*免疫球蛋白M(immunoglobulin M, IgM)阳性的检出率明显高于足月新生儿,说明母体的*Cr*血运感染可能未得到控制并暴露于胎儿,使胎盘-胎儿局部释放能够诱导子宫收缩和胎膜早破的炎症因子,导致流产或早产。在YEO等^[50]构建的小鼠模型中,皮下腔室注射*Cr*显著增加了胎儿吸收和宫内生长受限的发病率,并在母鼠的肝脏和胎盘中均检测到。OFFENBACHER等^[51]发现*Cr*感染引起胎盘炎症和蜕膜增生,这干扰了母鼠与胎鼠之间营养和代谢物的传递,增加了出生小鼠病死率,并使胎鼠脑部 γ -干扰素(interferon- γ , IFN- γ)的表达量显著增加;检测新生仔鼠的脑组织,观察到类似蛋白变性的细胞和髓鞘损伤;这些现象提示孕期母体感染*Cr*的风险不仅局限于妊娠期,还可能影响围产期神经的生长和发育。与*Fn*类似,*Cr*感染引起的炎症似乎也通过TLR4介导^[52]。此外,皮下腔室注射*Cr*引起小鼠胎盘印记基因*Igf2*启动子区域甲基化水平增高^[53],通过抑制多个与生长和发育相关基因(包括许多印记基因)的表达导致胎儿生长受限^[54],这些发现丰富了牙周致病菌对胎盘-胎儿造成损伤的机制。

*Pg*作为“红色”复合体的重要成员,不仅是牙周炎的关键致病菌,也是导致APOs的风险菌。队列研究显示,患APOs女性的牙周袋和羊水或绒毛膜组织中均能检测到*Pg*^[55-56]。多项动物实验和体外实验揭示*Pg*和APOs之间的相关性并支持口腔致病菌可经血运途径感染子宫的致病模式。1994年,COLLINS等^[57]通过妊娠金仓鼠模型证实*Pg*可通过菌血症传播到胎盘-胎儿,并诱发死产和低出生体质量。LIN等^[58-59]通过皮下腔室注射的方式用*Pg*感染受孕小鼠,导致小鼠出现胚胎生长受限或吸收的症状,进一步分析显示这与胎盘IL-10水平的降低以及TNF- α 、

Th1细胞和Th2细胞比率的增加有关。AO等^[60]发现经口感染*Pg*能够诱导受孕小鼠出现早产和低出生体质量,进一步分析显示母体血浆中促炎细胞因子TNF- α 、IL-17、IL-6和IL-1 β 的水平显著升高,且小鼠胎盘表现出广泛的变性和局部坏死,免疫组化分析检测到*Pg*在胎盘中定植。INABA等和REN等^[61-63]分别开展了*Pg*感染人绒毛膜滋养层细胞系HTR-8/Svneo的研究,发现*Pg*可通过激活ERK1/2、p38和JNK信号通路诱导细胞凋亡和G1期阻滞,还可诱导HTR-8/Svneo细胞分泌细胞因子IL-8和IFN- γ 。牙龈蛋白酶是*Pg*的重要毒力因子,可诱导宿主牙周组织和防御机制的破坏,并与细菌表面蛋白、分泌蛋白的加工以及血红素、氨基酸等营养物质的获取有关^[64]。TAKII等^[65]通过经静脉注射*Pg*的受孕小鼠模型证实*Pg*牙龈蛋白酶具有促进APOs的作用;与野生型相比,牙龈蛋白酶缺陷的*Pg*造成相对较低的早产率及较高的幼崽存活率和出生体质量,用牙龈素抑制剂治疗可预防由*Pg*感染引起的胎儿死亡和早产,并可恢复因*Pg*感染引起的IFN- γ 抑制。随着现在研究技术的发展,相信将会有更多牙周致病菌与APOs的关系被发现,更细致的致病机制和致病模式被阐明。

3.2 促炎介质引发宫内炎症反应失衡

妊娠期间,子宫内的先天性免疫反应受到严格调控,以维持精细的免疫稳态,从而防止携带父方DNA的胎儿诱发母体产生免疫排斥,促炎介质的局部增加会干扰这种平衡,进而引发炎症负担,造成APOs^[66]。口腔致病菌释放的内毒素可诱导宿主产生细胞因子,后者介导的促炎介质PGE2造成绒毛膜中前列腺素水平升高,导致宫颈成熟、子宫收缩和胎膜早破,增加流产或早产的风险。

动物模型研究提供了该致病途径的系列证据(表2)。例如, COLLINS等^[67]构建了孕前和孕中的金仓鼠模型,静脉注射*Pg*或*Escherichia coli*的脂多糖(lipopolysaccharide, LPS)造成畸胎和胎儿吸收的症状,且症状发生的比例和严重程度是LPS剂量依赖性的。在妊娠大鼠模型中,颈静脉注射*Pg*-LPS增加了大鼠的收缩压和胎儿吸收的风险,剂量依赖地降低了胎儿、胎盘质量,但并没有引起蛋白尿或全身炎症反应,在未受孕大鼠中则观察不到*Pg*-LPS的

表 2 牙周致病菌介导促炎介质引发宫内炎症反应失衡相关文献总结

Table 2 Summary of research reports on the imbalance of intrauterine inflammatory response caused by proinflammatory agents

Experimental method	Conclusion and importance
Cell experiment ^[56, 60]	Suggest that LPS of periodontal pathogens can increase the risk of APOs by causing imbalance of intrauterine inflammation
Animal experiment ^[67]	
Animal experiment ^[68]	Suggest that LPS of periodontal pathogens has a specific toxic effect on pregnancy

LPS: lipopolysaccharide; APOs: adverse pregnancy outcomes.

作用,这说明Pg-LPS对妊娠有特定的毒力效应^[68]。体外实验结果显示Pg-LPS通过TLR2介导的信号通路诱导人绒毛膜源细胞产生炎症因子IL-6和IL-8^[56];用Pg-LPS刺激HTR-8/Svneo细胞后,环氧化酶-2、IL-8和TNF- α 的水平以NF- κ B信号通路依赖的方式表达增加^[60](表2)。

此外,牙周致病菌或其致病组分及细胞因子从感染的牙周组织释放到血液循环中,通过肝脏的急性反应导致CRP表达水平升高引起全身性炎症,是该途径导致APOs的重要致病机制之一^[69]。人群研究的结果提示,牙周炎导致的孕妇血浆CRP水平升高与早产和先兆子痫有关^[70-73],其中一项来自印度的报道显示,妊娠期间的牙周治疗降低血浆CRP水平,且治疗后早产发病率显著下降,由治疗前的31.7%〔早产与足月产的优势比(odds ratio, OR)为1.97, 95%置信区间(confidence interval, CI): 1.14 ~ 3.39〕降为治疗后的15%(早产与足月产的OR为0.49, 95%CI: 0.27 ~ 0.88)^[72]。与致病菌通过菌血症由口腔至子宫的传播途径相比,牙周致病菌及相关炎症介质通过血液循环介导的肝内炎症途径尚缺乏系统的实验研究证据。可通过病例对照研究或孕期女性人群队列跟踪随访研究、验证牙周致病菌相关炎症与APOs之间的关系,发现更多可预测APOs的炎症标志物或免疫指标,通过动物实验和细胞实验进一步阐明该途径的具体致病机制,进而丰富和完善该领域研究的理论基础。

3.3 生殖道致病菌上行引发宫内感染

临床研究发现,大多数宫内感染源于下生殖道中病原微生物或其毒力因子经生殖道上行进入子宫,通常先感染羊膜和胎盘,进而通过脐带血流或吸入污染的羊水感染胎儿^[33]。因此,有科学家提出牙周致病菌可能由口腔经孕妇生殖道上行感染进入羊膜腔,这是牙周致病菌诱导APOs的第三种途径^[74-75],但目前仅有限的病例研究证据支持这一途径。1994年, DIXON等^[76]发表的一项病例报告中指出,在一名早产伴临床绒毛膜羊膜炎患者的羊膜腔中分离出了常见的牙周致病菌*Fn*和*Capnocytophaga* sp., 并且其伴侣有牙周炎史,问诊记录提示患者出现的妊娠并发症可能是伴侣的牙周致病菌接触生殖道后上行感染所致。之后,陆续有多项病例研究提示多种口腔共生菌/致病菌可能通过生殖道上行传播引发宫内感染^[77-80]。此外,多项研究提到APOs与女性生殖道中检出的致病菌密切相关,其中部分致病菌也是常见的口腔致病菌。目前,该领域的机制研究罕有报道,从生态学的角度来看,阴道生态环境与口腔生态环境相比有相似性又相对独特,而菌群的变化需要外源物种持续高剂量地入侵或剧烈扰动,牙周致病菌通过生殖道内定植的广泛性和普遍

性并不明晰^[34]。此外这些细菌本身就是生殖道常驻菌还是经口腔传播至此亦不清楚。未来,该领域的研究需要更多的临床病理分析、人群队列研究及动物模型研究,进而丰富和完善我们对该途径的可行性、普遍性及具体致病模式的认识。

4 牙周炎导致APOs的研究面临的挑战

宫内感染对孕妇自身以及妊娠结局的影响与胎龄、孕妇的免疫状态、所感染病原微生物的种类和剂量及感染的时间点和严重程度有关,是造成流产、死产、胎儿畸形和低出生体质量等APOs的重要原因^[20]。目前,生殖道病原微生物入侵是宫内感染的主要病因。口腔微生物及其炎性介质经血运传播导致宫内炎症反应的人群研究及动物模型研究揭示牙周致病菌通过血液传播等途径引起宫内感染是APOs的重要致病途径,已引起越来越多妇产科专家的重视。

目前,已鉴定出口腔中存在近800种微生物,但仍有很多菌株尚未被培养或鉴定出^[81]。复杂的口腔菌群可能有多种口腔传播到子宫的方式和模式,口腔病原菌物种间的相互影响在促进APOs发病机制中的作用仍罕有报道,口腔微生物在宫内感染中的作用范围很可能仍被低估。在2010年, FARDINI等^[82]利用动物模型研究了口腔菌群通过血运传播向胎盘转移的能力,通过分别将健康志愿者的唾液和牙周炎患者的龈下菌斑样本从尾静脉注射到受孕小鼠体内,模拟在牙周感染期间经常发生的菌血症病况,使用16S rRNA基因测序检测定植于小鼠胎盘的细菌种类,发现多种口腔细菌能够通过血液传播至小鼠胎盘,且这些物种中大多数是已报道的、可从人类APOs患者胎盘中检测到的病原菌。当给受孕小鼠注射PBS时,胎盘中检测不到细菌^[41],这说明能够在胎盘中检出的物种都源自唾液或龈下菌斑。值得关注的是,某些物种在胎盘中的丰度高于唾液或龈下菌斑,这提示口腔微生物在胎盘中的定植具有物种特异性,而不是以“随机扩散”的形式进入胎盘,该研究首次证实了宫内感染中存在特定的口腔细菌多样性和偏好性^[82]。

近年来,高通量组学分析技术促进了人体微生态领域研究的快速发展。健康的胎盘-胎儿长久以来被认为是无菌的,但2014年AAGAARD等^[83]发现胎盘中存在一个独特的微生态系统,且胎盘微生物组与人类口腔微生物组最接近。存在于胎盘和胎儿器官中的微生物群被认为对于胎儿出生前免疫功能的建立和启动具有重要意义^[84]。部分组学分析指出,患APOs妇女胎盘微生物群的组成与正常妊娠妇女相比发生了显著变化^[85-87]。然而,多项研究

对胎盘及羊膜微生物群的存在与否提出了质疑,认为此前有关胎盘微生物组的研究未能充分控制污染、缺乏严谨且适当的阴性对照^[88-89]。

胎盘-胎儿组织是否存在微生物群的争议为牙周炎对APOs影响的研究带来了新的机遇和挑战。结合已有的实验证据,BOBETIS等^[36]在2020年发表的综述中建议用来自牙周病学的“关键病原体”和“多微生物协同作用”理论来解释牙周炎导致APOs的原因,笔者也认同这一观点。在APOs相关的牙周炎“关键病原体”中,*Fn*是研究最多的物种,也是迄今宫内感染中发现的唯一梭杆菌属(*Fusobacterium*)物种。如前章3.1所述,毒力因子FadA在*Fn*定植胎盘的过程中发挥重要作用。FadA仅在*Fn*、*F. periodonticum*和*F. simiae*三种口腔梭杆菌中表达^[42],在其他梭杆菌属物种中都不存在。据报道,下生殖道最常见的梭杆菌物种*F. naviforme*和*F. gonidiaformans*未从羊水中分离出来^[90],FadA的缺失可以解释它们不能对子宫造成上行感染的原因。目前,研究显示口腔中的*Fn*可通过菌血症造成宫内感染,但有的研究显示牙周炎引发的菌血症和牙周炎的临床参数(如牙周袋深度)并无相关性^[91]。因此,对牙周炎影响APOs作用机制的研究应该更多地聚焦筛选“关键病原体”及其主要毒力因子的发掘与探索,以及研究病原体在致病过程中的相互作用,而不局限于牙周炎严重程度与APOs病理指标的对应关系分析。

牙周炎临床干预对APOs防治的不确定性是另一个问题。例如,BOBETIS等^[36]分析、总结了在全球各地开展的共计15项针对妊娠中期牙周非手术干预对APOs影响的人群随机对照试验,结果显示大多牙周干预仅改善了牙周炎相关参数,但对APOs的发生无显著影响。这一结果的合理解释是妊娠期间开展的牙周治疗不能清除妊娠早期已经进入胎盘-胎儿的口腔致病菌,或者牙周治疗没能缓解“关键病原体”造成宫内感染途径的暴露,因此牙周健康指标的改善未能有效缓解APOs症状。如何有效干预牙周炎引起的APOs风险仍需更多思考和创新型实践。

5 总结与展望

在研究牙周炎对APOs影响的近30年时间里,科研人员似乎从未质疑过这两种疾病的关系,但由于每种疾病本身复杂的病因以及各种外在干扰因素的影响,牙周炎导致APOs的途径和致病机制尚未完全清楚。总结、分析现有的研究结果,我们认为口腔致病菌经血运传播进入胎盘-胎儿引发宫内感染是牙周炎造成APOs的主要途径。鉴于牙周炎相关病理参数与APOs之间关系的不完

全对应,未来的研究应致力于发掘和解析APOs相关的口腔致病菌及其特定的毒力因子,以及解析致病菌间的相互作用和它们暴露于胎盘-胎儿的途径及致病模式。至于牙周治疗对妊娠的影响,鉴于已知口腔致病菌(如*Fn*)经血运传播途径感染的妊娠(时间)和胎盘(位点)特异性^[41],我们认为相较于妊娠期的干预,备孕期间的牙周治疗能够更有效地阻止口腔病原的胎盘-胎儿暴露。此外,当前许多牙周炎与APOs相关性研究的证据来自动物模型或体外实验,这可能不完全适用于人体内的实际情况。因此,通过人群研究,特别是高入组质量、全体检指标数据、预先排除干扰因素的前瞻性队列跟踪分析来获得确信的研究结果,对于全面解析牙周炎对APOs的影响范围和特点及制定针对性的干预策略以促进妊娠期的顺利进行是非常重要的。

* * *

利益冲突 所有作者均声明不存在利益冲突

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