

多囊卵巢综合征颗粒细胞线粒体功能障碍的研究进展

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[摘要] 多囊卵巢综合征(PCOS)患者存在卵泡发育和成熟障碍。卵巢颗粒细胞在原始卵泡的启动和生长发育过程中发挥着重要的调控作用, 其中颗粒细胞的线粒体参与了细胞周期、代谢及信号转导等方面的调节, 并通过能量代谢途径为卵母细胞减数分裂、受精直至早期胚胎发育提供能量支持。研究颗粒细胞的线粒体功能是探索PCOS的病理机制、评价卵母细胞质量和胚胎发育潜力的最佳无创性方法之一。大量证据显示, PCOS与卵巢颗粒细胞线粒体功能障碍相关, 其机制包括线粒体DNA(mtDNA)拷贝数改变和mtDNA基因突变等。有研究从改善PCOS颗粒细胞的线粒体功能入手, 对改善PCOS卵泡发育和成熟障碍进行了探索。该文对近年来PCOS颗粒细胞线粒体功能障碍的相关研究进展进行综述, 以探讨PCOS颗粒细胞线粒体功能障碍的发生机制及其改善途径和方法。

[关键词] 多囊卵巢综合征; 颗粒细胞; 线粒体; 卵泡发育障碍

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Research progress on mitochondrial dysfunction of granulosa cells in polycystic ovary syndrome

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[Abstract] Patients with polycystic ovary syndrome (PCOS) have impaired follicular development and maturation. Ovarian granulosa cells play an important regulatory role in the initiation, growth and development of primordial follicles, in which the mitochondria of granulosa cells are involved in the regulation of cell cycle, metabolism, and signal transduction, and provide energy support for oocyte meiosis, fertilization, and up to early embryonic development through energy metabolic pathways. Studying the mitochondrial function of granulosa cells is one of the best non-invasive methods to study the pathological mechanisms in PCOS patients and to evaluate oocyte quality and embryonic developmental potential. A large number of evidences have shown that PCOS is associated with mitochondrial dysfunction in ovarian granulosa cells by mechanisms including altered mitochondrial DNA (mtDNA) copy number and mutations in the mtDNA gene. Some studies have explored the improvement of mitochondrial function in PCOS granulosa cells to improve the impaired follicular development and maturation in PCOS. Therefore, the relevant research progress in recent years have been reviewed in present paper about mitochondrial dysfunction in PCOS granulosa cells, for exploring the mechanism of mitochondrial dysfunction in PCOS granulosa cells, and the ways and methods of improvement.

[Key words] polycystic ovary syndrome; granulosa cell; mitochondria; follicular dysplasia

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PCOS)是育龄女性常见的内分泌、代谢紊乱性疾病, 其对生殖力的影响贯穿于整个生育期^[1], 临床上主要表现为卵泡发育异常、排卵障碍及流产等^[2-3], 根据不同的人群及诊断标准, 其患病率为5%~15%^[4]。PCOS是无排卵性不孕的最常见病因, 占无排卵性不孕的70%^[5]。PCOS患者的卵泡发育障碍主要表现为窦前卵泡过多、窦状卵泡生长障碍及

卵泡闭锁等。患者双侧卵巢虽有大量窦卵泡，但不能周期性形成优势的成熟卵泡是导致不孕的重要原因。同时，PCOS患者行体外受精移植(*in-vitro fertilization-embryo transfer*, IVF-ET)时，虽然获卵数目明显增加，但卵母细胞质量通常不佳，从而导致移植周期取消率高及受精率低^[6]。

全基因组关联分析(*genome wide association study*, GWAS)及对核基因组进行的其他研究未能成为PCOS发病的精确机制提供相关线索，而越来越多的研究发现，线粒体功能障碍可能是卵泡发育障碍的重要原因之一^[7-8]。本文对近年来PCOS颗粒细胞线粒体功能障碍的相关研究进展进行综述，以探讨PCOS颗粒细胞线粒体功能障碍的发生机制及其改善途径和方法。

1 线粒体功能与卵泡发育

线粒体是重要的细胞器之一，含量较为丰富，是细胞代谢的重要场所。线粒体由内膜、外膜、膜间隙及基质等构成。其中，线粒体内膜深度内陷形成线粒体嵴，为线粒体中各种呼吸链复合物及各种脂类合成代谢相关酶提供附着位点及反应场所。线粒体在决定生殖过程的众多因素中起关键作用，如卵母细胞质量、卵泡生长发育和颗粒细胞增殖等^[9]。线粒体还介导多种细胞过程，包括细胞凋亡、活性氧簇(*reactive oxygen species*, ROS)调控、钙信号传导，以及三磷酸腺苷(*adenosine triphosphate*, ATP)、嘧啶、铁硫蛋白(*iron sulfur proteins*, Fe-S)的合成等^[10]。

线粒体DNA(*mitochondrial DNA*, mtDNA)是独立于细胞核之外的遗传物质，可以完整地传达遗传信息。mtDNA是一种具有16 569个碱基对的闭合双链环状分子，编码22个转运RNA(*tRNA*)，以及独立的D-loop区，两个核糖体RNA(*rRNA*, 12S和16S)和13个氧化磷酸化蛋白亚单位。mtDNA损伤或突变都可能诱导线粒体功能障碍，从而影响能量的产生以及组织、器官的功能^[11]。

线粒体是卵母细胞质中含量最为丰富的细胞器，几乎提供了卵母细胞生命活动中的全部能量。线粒体是胚胎内数量最多的母系遗传细胞器，胚胎中的线粒体全部来自于卵母细胞，卵子内线粒体的数量及功能与胚胎质量密切相关^[12]。

线粒体在颗粒细胞中的含量也非常丰富，参与了颗粒细胞的细胞周期、代谢及信号转导等方面的调节^[13]。颗粒细胞线粒体可通过能量代谢途径为卵母细胞减数分裂、受精直至早期胚胎发育提供能量支持^[14]，也为甾体激素如孕酮、雄激素、雌激素等的合成提供能量支撑^[15]。线粒体发生异常可影响细

胞的正常功能，甚至诱导细胞死亡。有学者对3名妇女冻存的卵巢组织原始卵泡和初级卵泡中的颗粒细胞转录组进行类比研究发现，在来自原始卵泡的颗粒细胞中，包括编码自由基清除剂过氧化物还原酶5(*PRDX5*)及硫氧化还原蛋白2(*TXN2*)等在内的30个基因与线粒体功能障碍相关，提示原始卵泡颗粒细胞发育过程中有大量的能量产生和自由基清除，其线粒体功能障碍将影响原始卵泡的进一步发育，推测这可能是PCOS形成的潜在机制之一^[16]。

2 卵巢颗粒细胞与卵泡发育

卵母细胞、颗粒细胞和卵泡膜细胞是卵泡的主要组成部分。卵泡发育是一个复杂的生理过程，颗粒细胞的生长分化是其重要标志。生长期卵泡的发育及卵泡闭锁是通过受体介导途径调控的，颗粒细胞自分泌及旁分泌的物质可促进颗粒细胞增殖及卵泡生长^[17]，同时颗粒细胞与膜细胞的相互作用是卵泡发育和维持正常功能的重要条件，因此，颗粒细胞在原始卵泡的启动和生长发育中起着重要的调控作用^[18]，颗粒细胞代谢、生长分化及凋亡率的改变与卵泡发育成熟或闭锁息息相关，甚至可能影响胚胎的质量及最终的受孕结果^[19]。有研究发现，颗粒细胞线粒体质量下降，及其结构、功能和mtDNA拷贝数量异常是卵泡发育障碍的重要原因之一^[20-21]。

3 卵巢颗粒细胞线粒体功能与卵母细胞质量

目前，在生殖医学中，多基于胚胎形态(如形态计量及形态分析等)及反映胚胎活力的发育动力学指标评估卵母细胞质量^[22]。然而，上述评估标准的信息量非常有限，且很难在不改变其完整性的情况下进行研究，操作上仍存在局限性^[23]，因此，目前研究者正试图探索其他可靠的、非侵入性的可提示胚胎功能的生物标志物^[24]。

研究卵母细胞周围的微环境如壁层颗粒细胞、卵丘细胞、卵泡液，以及其与临床参数的相关性具有重要意义^[25]。人类在胎儿期就已形成始基卵泡储备，颗粒细胞自始基卵泡时期就围绕在卵母细胞周围，与卵母细胞处于同一卵泡环境中，两者之间通过紧密接触和缝隙连接进行物质交换及信息沟通，因而研究颗粒细胞的功能可以反映卵母细胞的发育和质量^[26-27]。卵母细胞在氧化磷酸化过程中合成ATP的底物丙酮酸盐即是由颗粒细胞提供的。丙酮酸盐是颗粒细胞葡萄糖糖酵解过程中产生的中间代谢物。由于颗粒细胞相对容易获取，且可间接反映卵母细胞质量，因此，着眼于卵巢颗粒细胞的研究被认为是评价卵母细胞质量和胚胎发育潜力的最佳无创性方法之一^[28-29]。

颗粒细胞线粒体作为能量代谢途径的关键因素,直接参与了卵泡发生过程中卵母细胞功能的建立^[13]。因此,线粒体功能对于颗粒细胞的能量生产,进而作为卵母细胞成熟的能量来源是至关重要的。将颗粒细胞线粒体作为评估卵母细胞质量的指标有一定的可行性及可操作性^[30]。

研究表明,PCOS患者卵母细胞有低糖酵解活性,并优先摄取颗粒细胞提供的能量底物来维持能量平衡^[31]。颗粒细胞功能失调可导致严重的细胞损伤,影响卵母细胞成熟,最终使PCOS患者生成异常卵泡^[32-33]及卵母细胞核成熟和受精率下降^[34]。因此,颗粒细胞对维持卵母细胞的稳态至关重要。有研究指出,在PCOS患者的卵泡发生过程中,卵母细胞从氧化磷酸化到糖酵解的代谢转化紊乱可归因于颗粒细胞的线粒体功能障碍^[32]。

4 PCOS颗粒细胞线粒体功能障碍的发生机制

4.1 mtDNA拷贝数改变

PCOS患者mtDNA拷贝数异常及线粒体基因突变一直是研究的重点,而功能性线粒体疾病已逐渐被视为PCOS发病的相关因素^[35]。

mtDNA拷贝数是衡量线粒体数量的相对指标。维持mtDNA拷贝数对于保持线粒体功能和细胞生长至关重要^[36]。在卵母细胞成熟过程中,mtDNA不断复制,发育至成熟卵母细胞时其拷贝数已增加30倍以上^[37],通常包含至少 1×10^5 个mtDNA拷贝,这是正常卵泡发育和成熟所必需的^[38]。

Wang等^[39-40]研究糖尿病小鼠间接提示了周围颗粒细胞mtDNA与卵母细胞具有相关性;同样的结果也在猪模型的研究中得到验证^[41-42]。在对人类的研究中已证实颗粒细胞mtDNA含量与相应卵母细胞的mtDNA含量、胚胎质量之间存在相关性,提示颗粒细胞可能通过提高卵母细胞质量,改善卵泡发育,从而支持足够的胚胎发育^[43-44]。mtDNA拷贝数可评估卵母细胞和胚胎发育的潜能^[45-46]。

线粒体拷贝数目前多采用实时荧光定量聚合酶链反应(qRT-PCR)进行检测,后者是一种敏感且较为成熟的检验技术^[47-48]。Reddy等^[33]发现在PCOS女性中mtDNA拷贝数明显减少。而mtDNA拷贝数减少可导致mtDNA编码基因的线粒体功能障碍,使ROS生成增加及PCOS进一步发展^[3,49]。Lewis等^[50]发现,PCOS患者的mtDNA拷贝数较正常女性低,且mtDNA拷贝数与胰岛素抵抗(insulin resistance, IR)水平、腰围和三酰甘油水平呈负相关,与性激素结合球蛋白(sex hormone-binding globulin, SHBG)水平呈正相关。Ogino等^[51]通过检测卵丘颗粒细胞mtDNA拷贝数对体外受精过程中的胚胎质量进行

预测,其阳性预测值和阴性预测值分别为84.4%和82.1%,因此提出颗粒细胞mtDNA可作为预测胚胎存活率的生物标志物。

4.2 mtDNA突变

mtDNA较核DNA(nucleus deoxyribonucleic acid, nDNA)更容易受到氧化损伤,并存在更高的突变率,可能的原因包括mtDNA的表达、维持、拷贝数调节及修复过程依赖于核基因组^[52],缺乏自身保护组蛋白,缺乏有效的DNA修复能力,以及与电子呼吸链关系密切而接触到了更多的氧自由基等^[33]。

线粒体转运RNA(mitochondrial transfer RNA, mt-tRNA)的突变破坏了mtDNA的结构和功能,影响了mt-tRNA的稳态水平和氨基酰化能力,可能导致线粒体蛋白质合成缺陷,从而抑制呼吸链功能,并导致ATP合成减少和ROS生成增加^[53],可能与PCOS患者线粒体功能障碍的发生机制有关。Goto等^[54]在PCOS患者外周血中发现mt-tRNA基因、12S和16S rRNA基因存在变异,这些突变出现在高度保守的tRNA核苷酸中,因此提出mtDNA编码mt-tRNA的基因点突变可能与疾病的发展有关。Ding等^[55]对中国一个有遗传性IR的汉族家系进行了研究观察,对其线粒体进行基因组序列分析,此家族中第三代为PCOS患者,全线粒体基因组序列分析结果显示,在tRNA^{Leu}(UUR)基因的受体臂中存在同源A3302G,此突变破坏了高度保守的碱基配对(2T-71A)。随后该团队继续对该家系进行线粒体基因组序列分析,发现存在同质ND5 T12338C和tRNA^{Ser}(UCN) C7492T突变,其中同源C7492T突变发生在tRNA^{Ser}(UCN)编码基因的反密码子干26位^[56],影响了tRNA的稳态水平和氨基酰化能力;之后再通过对80例PCOS合并IR女性患者与健康对照组进行mt-tRNA突变检测,筛选出可能与PCOS合并IR相关的9个mt-tRNA突变,提出这些突变改变了mt-tRNA的二级结构,从而导致mt-tRNA功能障碍^[57]。Ding等^[58]发现,PCOS患者存在线粒体基因tRNA^{Leu}中的C3275T突变,tRNA^{Gln}的反密码子干中的T4363C突变,以及tRNA^{Lys}的A8343G突变;生化分析结果显示,这些mt-tRNA突变患者的线粒体膜电位(mitochondrial membrane potential, MMP)水平、ATP产量及mtDNA拷贝数均降低,但ROS生成增加,提示这些突变可能导致线粒体功能障碍,进而出现相应的临床表现。Saeed等^[49]对PCOS患者的mt-tRNA进行测序并与修正剑桥序列进行比对,证实了上述观点,且新发现了mt-tRNA^{Leu}(UUR)基因不同类型的突变。

mtDNA D-loop区是线粒体基因组中唯一的非编码区,包含了mtDNA重链和轻链复制的起点和

启动子；该区域突变可通过改变mtDNA的复制和转录，而使线粒体功能发生障碍^[59]。Reddy等^[33]对南印度PCOS患者与对照组的线粒体D-loop进行测序，发现D310及A189G单核苷酸多态性(SNPs)与PCOS之间存在明显的相关性，携带D310和A189G等位基因的PCOS患者mtDNA拷贝数明显低于非携带者。

这些结果表明，PCOS的临床表型可能与上述突变引起的线粒体功能障碍有关，但要进一步探讨mt-tRNA异常的临床意义，阐明mt-tRNA突变与PCOS之间的关系，仍需进行多种族和多中心的深入研究。

5 改善PCOS颗粒细胞线粒体功能的方法

已有团队在此方面进行了尝试和探索。Safaei等^[60]对脱氢表雄酮(dehydroepiandrosterone, DHEA)诱导培养的PCOS小鼠颗粒细胞研究发现，采用维生素D₃治疗的PCOS小鼠中，线粒体生物起源基因过氧化物酶体增殖物激活受体 γ 共激活因子1 α (peroxisome proliferator-activated receptor- γ coactivator-1 α , PGC-1 α)、核呼吸因子(nuclear respiratory factors, NRFs)、抗氧化剂基因超氧化物歧化酶(SOD)、谷胱甘肽过氧化物酶(GSH-Px)、过氧化氢酶(CAT)及抗凋亡基因Bcl-2表达上调，同时ROS水平降低，提示维生素D₃可能通过丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)信号通路来改善线粒体的生物起源基因。此外，该团队还发现，经维生素D₃处理的PCOS小鼠颗粒细胞mtDNA拷贝数明显高于未经处理的PCOS颗粒细胞，提示维生素D₃可能通过促进PCOS小鼠颗粒细胞线粒体生物合成及细胞膜完整性，增加mtDNA拷贝数，从而改善卵泡发育，进而提高卵母细胞的质量^[61]。

Salehi等^[62]对二氢睾酮(dihydrotestosterone, DHT)诱导培养的PCOS大鼠颗粒细胞进行研究，发现，DHT诱导的颗粒细胞动力蛋白相关蛋白1(Drp1)表达上调与PCOS大鼠颗粒细胞线粒体过度裂变、巨自噬及细胞凋亡有关，而外源性促性腺激素可诱导线粒体融合并减轻颗粒细胞的凋亡。

中医药是中华民族瑰宝，中药可通过多途径、多靶点缓解PCOS患者的临床症状，改善卵巢内环境，促进卵泡生长发育，诱发排卵。苍附导痰汤可通过抑制miR-29a靶向结合PGC-1 α 的3'端非翻译区(3'-UTR)，促进PGC-1 α 及其下游的核呼吸因子-1抗原(nuclear respiratory factor-1, NRF-1)、雌激素受体相关受体 α (estrogen-related receptor α , ERR α)的mRNA表达，从而促进线粒体生物合成、

葡萄糖利用、脂肪酸氧化，并改善PCOS-IR大鼠卵巢颗粒细胞的IR状态^[63]。

6 总结与展望

综上所述，PCOS患者存在线粒体功能障碍及基因表达异常，而线粒体在生殖细胞中含量丰富，且相对容易获取，故研究颗粒细胞的线粒体功能是探讨PCOS的病理机制、评价卵母细胞质量和胚胎发育潜力的最佳无创性方法之一。然而，在PCOS患者颗粒细胞线粒体功能障碍的具体机制、与临床表型之间的关系，以及筛选评估预后的特异性指标等方面，目前尚缺乏系统研究，仍有待进一步探索。

在治疗方面，线粒体替代或线粒体补充疗法用于临床改善PCOS的卵母细胞及胚胎质量是否可行，因伦理争议和异质性风险无法开展。目前，我们可以发挥中医药的优势，采用现代药理学研究方法，探讨中药对PCOS颗粒细胞线粒体功能的调节作用，这对PCOS的治疗具有重要的指导意义，也是中医药走向现代化的探索之路。

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