

抗氧化剂在不孕不育症治疗中的应用

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不孕不育是指有生育意愿的男女双方同居 1 年以上, 有正常性生活且未避孕, 但未能建立或维持临床妊娠^[1]。世界卫生组织 (WHO) 调查显示, 目前不孕不育已成为仅次于肿瘤和心脑血管疾病之后的第三大疾病。流行病学调查显示, 世界范围内育龄夫妇不孕不育发病率高达 8%~12%, 且呈逐年递增趋势, 给社会和家庭带来沉重的心理和经济负担^[2,3]。研究显示, 适量浓度的活性氧 (reactive oxygen species, ROS) 在生殖过程中具有重要作用, 可影响卵泡生长、卵母细胞成熟、精子发生、精卵结合、胚胎着床和胚胎发育等^[4]。但是, 过量的 ROS 会造成氧化应激, 引起细胞毒性并损伤 DNA, 进而导致不孕不育的发生^[5]。因此, 抗氧化剂有望改善由于氧化应激而导致的不孕不育, 本文将详细阐述氧化应激如何诱发不孕不育, 以及常见抗氧化剂在不孕不育症治疗中的应用。

1 氧化应激与不孕不育

1.1 氧化应激对女性生育力的影响

近年来, 国内外越来越多的学者开始关注氧化应激对女性生育力的影响。研究提示, 细胞色素 P450 可影响 ROS 的产生, 黄体本身也是 ROS 主要来源。卵母细胞成熟过程包括减数分裂 I 和 II, 在很大程度上受 ROS 浓度和抗氧化剂的影响^[6]。研究显示, 在排卵过程中, 随着促黄体生成素 (luteinizing hormone, LH) 的激增, 卵泡内会新生大量血管, 导致血管内皮细胞和巨噬细胞产生大量 ROS, 卵泡内适量的 ROS 有利于卵母细胞成熟和卵泡破裂^[7]。ROS 被认为是排卵的关键诱导因子, 抑制

ROS 已被证实会干扰排卵^[8]。但是, 过量的 ROS 则会导致卵巢中氧化剂和抗氧化剂的失衡, 进而诱发氧化应激损害卵母细胞和颗粒细胞, 导致卵母细胞质量差。

此外, 氧化应激通过引起细胞膜脂质过氧化、蛋白质变性和 DNA 损伤, 对卵母细胞、胚胎发育和胚胎植入都有直接影响^[9]。氧化应激也与卵巢早衰、子宫内膜异位症、多囊卵巢综合征 (polycystic ovary syndrome, PCOS) 和不明原因的不孕有关^[8,10]。

1.2 氧化应激对男性生育力的影响

研究发现, 适量浓度的 ROS 是精子获能、超激活和顶体反应所必需的^[11]。然而, 高水平 ROS 造成的氧化应激不仅会导致精子脂质过氧化和 DNA 损伤, 还会导致精子中酶的失活和蛋白质变性, 降低精子活力, 从而降低受精率和妊娠率, 与男性不育存在密切关联^[12]。产生氧化应激的来源很多, 包括精索静脉曲张、抽烟、饮酒、肥胖/代谢综合征、白细胞精子症、性传播疾病 (即淋球菌、沙眼衣原体、梅毒螺旋体) 以及病毒感染 (如人类免疫缺陷病毒、肝炎) 等^[13]。

相比体细胞, 精子更易受到氧化应激损伤。在体细胞中, 抗氧化酶广泛存在于细胞质中, 然而在精子发生的最后阶段, 精子细胞质被移除, 导致精子只能依赖于富含抗氧化剂的精浆^[14]。此外, 精子细胞膜富含多种不饱和脂肪酸, 这使得它们更容易受到 ROS 诱发的脂质过氧化, 细胞膜的损伤将导致精子活力降低^[15]。多项研究证明, 不育男性精液中 ROS 水平明显高于可育男性, 抗氧化剂浓度则呈现较低水平^[16]。

2 抗氧化剂的分类

抗氧化剂是过量 ROS 的“清道夫”,有助于维持机体氧化剂/抗氧化剂的动态平衡。抗氧化剂主要分为两种类型:酶类抗氧化剂和非酶类抗氧化剂^[17]。酶类抗氧化剂主要包括超氧化物歧化酶(superoxide dismutase, SOD)、过氧化氢酶(catalase, CAT)、谷胱甘肽过氧化物酶(glutathione peroxidase, GPx)和谷胱甘肽还原酶(glutathione reductase, GR);非酶类抗氧化剂主要包括谷胱甘肽(glutathione, GSH)、褪黑素、辅酶 Q10(coenzyme Q10, CoQ10)、N-乙酰半胱氨酸(N-acetylcysteine, NAC)、维生素 C 和 E、肉碱、锌、硒、番茄红素等^[18]。

3 抗氧化剂在女性不孕症治疗中的应用

多项研究证实,抗氧化剂治疗可有效改善女性生育力:降低雄激素水平和胰岛素抵抗、提高卵巢储备功能、促进子宫内膜血液循环等,对于卵巢早衰、卵巢低反应、PCOS 和子宫内膜异位症具有一定的效果^[10,19]。

3.1 酶类抗氧化剂

SOD 是体内最有效的抗氧化酶之一,也是身体抵抗 ROS 的第一道防线。SOD 可以将超氧阴离子自由基转化为 H_2O_2 ,然后通过 GPx 或 CAT 将 H_2O_2 转化成 H_2O 和 O_2 ,从而防止过量氧自由基对各种细胞组分的毒害损伤^[9]。

以往报道显示,所有抗氧化酶在女性生殖过程中都起着关键作用。研究发现,在初级卵泡和次级卵泡的卵泡液中,SOD 的 3 种亚型(SOD1、SOD2 和 SOD3)浓度和活性均高于成熟的窦卵泡^[20-22]。这些研究提示,窦卵泡卵泡液中 SOD 活性相对降低,这对于确保排卵前所需要的 ROS 阈值水平非常重要。LH 是垂体前叶分泌的促性腺激素,对于女性正常排卵和黄体发育至关重要。一项研究表明,LH 诱导黄体中抗氧化酶 SOD1、SOD2 和 CAT 在 mRNA 和蛋白质水平表达均上调^[23]。

也有研究证实,当体外添加卵泡刺激素培养山羊颗粒细胞时,CAT 的活性显著提高,同时雌二醇分泌也显著升高。提示 CAT 可能在卵泡发育过程中发挥功能性作用^[24]。研究显示 CAT 存在于小鼠卵母细胞核中,其形成独特的囊泡结构以保护基因组免受减数分裂成熟过程中的氧化损伤,抑制 CAT 活性可增加内源性 ROS 水平,导致卵母细胞核染色体缺陷和 DNA 损伤^[25]。临床研究发现,GPx 在受精卵子卵泡液中的活性明显高于未受精卵子卵泡液,证明 GPx 在精卵结合过程中发挥重要作用^[26]。

综上所述,内源性酶类抗氧化剂能有效抵御氧化应激对卵巢及卵泡造成的损伤,从而维持女性正常生理和生殖功能。

3.2 非酶类抗氧化剂

GSH 是非酶类抗氧化剂的代表,广泛存在于内脏及生殖器官组织及细胞中。GSH 具有游离的巯基,因此具有很强的供电子或质子氢能力,可参与氧化还原过程,具有清除自由基、增强抗氧化物酶活性、提高机体抗氧化能力的功能^[27]。在雌性卵泡发育过程中,它可以保护卵子免受氧化损伤,也有研究表明,卵细胞内 GSH 水平的升高有益于胚胎发育^[28]。据报道,GSH 缺乏与卵巢早衰甚至与卵巢癌有关^[29]。另一项研究发现,对于接受体外受精的女性,女性卵泡中 GSH 水平较高会明显提高受精率^[30]。另外,研究发现 GSH 参与调节引起慢性炎

症的基因,这可能与自身免疫相关,提示 GSH 将来有望应用于免疫性流产和治疗抗精子抗体而导致的不孕患者^[27]。

褪黑素是由松果体分泌的具有较强抗氧化能力的神经内分泌激素,能够直接清除多种氧自由基,激活抗氧化酶的生成,提高电子传递链的效率,促进 ATP 合成^[31]。相比传统的抗氧化剂,如维生素 C 和 E、GSH 等具有更强的抗氧化潜力,成为近年来研究新热点^[32]。褪黑素既是脂溶性又是水溶性,可以轻易通过细胞膜^[33]。越来越多研究证明,褪黑素可直接作用于卵巢,促进卵泡发育、卵子成熟^[34]。研究显示,褪黑素可通过减低细胞核、线粒体和细胞膜的氧化应激,进而保护颗粒细胞结构完整性^[35]。体外研究显示,添加 10 μ M 褪黑素可有效提高促排卵周期中未成熟卵母细胞的发育潜能,最终获得高比例优质囊胚^[36]。最新的一项研究证实,褪黑素可以通过抑制氧化损伤和维持卵细胞膜通透性,提高人卵母细胞的冷冻和复苏效果,这为女性生育力保存提供了新思路^[37]。

CoQ10 是一种脂溶性抗氧化剂,可参与线粒体氧化磷酸化过程中的电子传递运输,保护细胞免遭氧自由基的攻击^[38]。有研究显示,高龄女性和老年小鼠卵母细胞中 CoQ10 浓度显著下降,线粒体 ATP 产生减少,通过膳食补充 CoQ10 可以进行逆转^[39]。一项随机对照研究显示,服用 CoQ10(600 mg/d,分为 3 次,持续 60 d)可以提高 IVF/ICSI 助孕过程中卵巢低反应女性的获卵率和受精率,并提高胚胎质量^[40]。同时,也有研究证明,将小鼠卵母细胞暴露于不孕患者卵泡液环境后,添加 CoQ10 可以提高小鼠卵母细胞的体外成熟^[41]。

维生素 C(抗坏血酸,水溶性)和维生素 E(α -生育酚,脂溶性)是最常见的维生素类抗氧化剂,可以中和并减少 ROS,进而降低氧化应激损伤^[8]。动物实验发现,脱氢表雄酮诱导的 PCOS 大鼠模型灌胃维生素 C(150 mg/kg,持续 15 d),显示卵巢组织中氧化应激产物显著降低,抗氧化酶(SOD 和 CAT)活性升高^[42]。研究显示,PM2.5 环境暴露可引起雌性小鼠抗苗勒管激素(anti-Müllerian hormone, AMH)水平降低和卵巢组织损伤,维生素 C(45 mg/kg/d)和维生素 E(100 mg/kg/d)补充均可以抑制由 PM2.5 引起的炎症和氧化应激^[43]。子宫内膜异位症与慢性炎症有关,ROS 则是调节细胞增殖的促炎介质,因此子宫内膜异位症可能与 ROS 有关^[44]。研究证实,NAC 和维生素 E 联合用药可以治疗子宫内膜异位症,二者可能通过抑制 NF- κ B 活性进而抑制子宫内膜异位细胞增殖^[45]。

近年来,国内外专家开始关注维生素 D 在女性生殖中的作用。大量证据表明,维生素 D 对 PCOS 和子宫内膜异位症患者可能具有关键作用,并改善其 IVF 妊娠结局^[46-47]。临床治疗一般使用复合维生素,队列研究表明,复合维生素可提高女性生育能力^[48-49]。最新的 Cochrane 系统评价报道了抗氧化剂对女性生育力的影响^[50],提示复合维生素可以提高临床妊娠率和活产率。CoQ10 可以活化维生素 C 和维生素 E,增强抗氧化能力。一项随机双盲临床试验结果显示,补充维生素 E(400 IU/d)和 CoQ10(200 mg/d),持续 8 周后可显著降低胰岛素抵抗和血清睾酮水平^[51]。

肉碱又被称为“准维生素”。左旋肉碱(L-carnitine, LC)和乙酰肉碱(acetyl-carnitine, ALC)均可通过改善线

粒体功能治疗不孕症^[52-53]。虽然这两种形式的肉碱都具有抗氧化性能,但一些报道显示,ALC 抵抗 ROS 诱导的氧化损伤方面比 LC 更有效^[54]。ALC 常用于抗氧化/抗衰老,而 LC 常用于促进脂肪燃烧和能量产生^[55]。最近的研究表明,PCOS 患者口服 LC (250 mg/d,持续 12 周)可显著降低体重、BMI 和腰臀比,并改善胰岛素抵抗^[56]。也有报道显示,功能性下丘脑闭经患者口服 ALC (1 g/d,持续 16 周),LH 水平显著升高,提示 ALC 对促性腺激素缺乏症不孕女性具有积极治疗作用^[57]。动物实验研究发现,在卵母细胞体外成熟培养体系中添加 LC,可减少细胞内 ROS 水平,进而提高卵母细胞和胚胎的发育潜能^[58]。

4 抗氧化剂在男性不育症治疗中的应用

多项研究证实,抗氧化剂治疗可有效改善男性生育力:增加精浆抗氧化能力、提高精子数量和精子活力、降低精子畸形率等,对治疗少弱畸形精子症等具有积极效果^[11,59]。

4.1 酶类抗氧化剂

研究显示,SOD 可保护精子免受脂质过氧化和氧化应激的侵害^[60]。Kobayashi 等^[61]发现,精浆中富含多种抗氧化酶,去除精浆的精子悬浮液在体外培养 120 min 后精子活力明显下降,氧化应激水平升高,添加 SOD (400 U/mL)可以有效降低氧化应激并提高精子活力。在人和大鼠精浆及精子细胞中均发现 CAT 的存在,其来源是前列腺,该酶在一氧化氮诱导的精子获能中起关键作用。精液中抗氧化系统的另一种酶是 GPx,也是来源于前列腺。它可以催化过氧化氢和有机过氧化物(包括磷脂过氧化物)还原,以降低 ROS 含量^[62]。在精子中,GPx 主要位于线粒体基质中,但是也发现了一种保护精子 DNA 免受氧化损伤并参与染色质浓缩过程的核形式^[63]。

4.2 非酶类抗氧化剂

研究发现,人类精浆中 GSH 减少导致精子中部结构异常,最终导致运动障碍^[64]。研究发现,GSH 补充可显著改善不育男性的精子参数(如数量、活动性和形态),GSH 还对细胞膜脂质损伤起着重要作用,其有效保护剂量为 600 mg/d,持续 2~3 个月^[65]。NAC 是 GSH 的前体,可以改善精液体积及黏稠度,并提高精子活力^[66]。NAC 最常用的剂量是 600 mg/d,一般与其他抗氧化剂联合使用至少 3 个月^[67]。动物实验也证实,NAC 可以保护精子 DNA 免受氧化损伤,改善小鼠精子参数^[68]。

最近的研究表明,褪黑素可以调节睾丸发育、减少睾丸损伤,并可用于临床男性生育力保存^[69]。精液冷冻保存是男性生育力长期保存的最佳方法,但是因为精子没有细胞质,冷冻过程中容易受到氧化应激影响,破坏精子质膜完整性并降低线粒体活性^[70]。近年来褪黑素作为精子冷冻保护剂被广泛研究,添加褪黑素可以上调几种抗氧化酶(SOD、CAT 和 GPx),从而降低 ROS 水平,保持精子膜的流动性和活力^[71]。有研究显示,冷冻保护剂中添加 0.1 mM 褪黑素,可提高抗凋亡基因 Bcl-2 和热休克蛋白 90 的表达,进而增强抵御氧化应激的能力^[72]。也有研究发现,在人精子冷冻保存中,最佳的褪黑素浓度为 3 mM,可以降低细胞内 ROS 水平,更好地保持精子活力^[73]。Neelam 等^[74]将雄性小鼠暴露于微波辐射

(900 MHz)35 d(每天 2 h),发现褪黑素(日剂量:5 mg/kg)可以显著降低睾丸组织中丙二醛和 ROS 浓度,改善微波辐射对精子数量、睾酮水平和 DNA 碎片化的影响。

CoQ10 在精子中段含量丰富,可以通过促进线粒体电子传递,进而促进精子的成熟和改善精子质量^[75]。Takeshima 等^[76]研究发现,CoQ10 可以再生其他抗氧化剂,例如维生素 C 和 E,进而提高其抗氧化能力。另外,CoQ10 可以有效降低氧化应激,避免精子 DNA 损伤,改善精索静脉曲张患者的精子参数^[77]。一项前瞻性研究提示,接受 CoQ10 治疗的男性不育患者(200 mg/d,持续 6 个月),精子浓度和活力显著升高,妊娠率和活产率没有明显改变^[78]。在一项随机双盲安慰剂对照试验中,口服 CoQ10 治疗特发性少精子症患者(200 mg/d,持续 3 个月),发现精浆中 CoQ10、GPx、总抗氧化剂能力显著提高,精子浓度和活力也显著上升^[79]。

类胡萝卜素是天然抗氧化剂,可保护细胞膜的完整性,调节上皮细胞的分化并有助于精子生成,研究证实,饮食中缺乏类胡萝卜素会降低精子活力^[62]。研究发现,维生素 A(视黄醇)的低血清水平会降低精子质量,推测维生素 A 可用于治疗男性不育症^[80]。人类精浆中维生素 C 含量约是血清中的 10 倍,研究证实维生素 C 的浓度与正常精子百分比呈正相关,与 DNA 碎片率呈负相关^[81]。临床研究发现,口服维生素 C 剂量 1 g/d,可以有效治疗男性不育症^[82]。维生素 E 可保护精子细胞膜免受氧化应激侵害,防止脂质过氧化^[62]。一项临床试验表明,每天摄入 600 mg 维生素 E,3 个月后精子功能得到改善^[83]。

2018 年一篇系统性 Meta 分析^[84],总计纳入 19 项回顾性研究和 10 项前瞻性随机对照研究(RCT),其中 26 项研究发现抗氧化治疗可以改善精液常规参数和辅助生殖助孕结局。本研究发现,抗氧化剂单一疗法或联合疗法最常用剂量如下:维生素 C(500~1 000 mg)、维生素 E(400 mg)、肉碱(500~1 000 mg)、NAC(600 mg)、CoQ10(100~300 mg)、锌(25~400 mg)、硒(200 mg)、叶酸(0.5 mg)、番茄红素(6~8 mg)。

然而,关于抗氧化剂是否可以治疗男性不育依旧存在争议。2020 年美国生殖医学学会对此进行了热点讨论,反对方 Steiner 教授认为口服抗氧化剂并不能改善精液质量,无益于男性不育者提高妊娠率^[85]。支持方 Chavarro 教授指出 2019 年 3 月 Cochrane 研究,涵盖 17 种抗氧化剂,发现抗氧化剂可提高男性不育患者子代的活产率^[86]。同时 Chavarro 教授也承认目前对于抗氧化剂的研究尚且有限,未来需要更深入的研究。

5 小结与展望

近年来,氧化应激被认为是导致不孕不育的主要原因。尽管正常生殖过程需要低水平的 ROS,但 ROS 水平过高会导致脂质过氧化和 DNA 损伤,进而干扰精卵功能。诸多研究表明,当几种抗氧化剂组合使用时,可以有效抵御氧化应激,改善生殖功能。然而,关于这些抗氧化剂的剂量和使用时间尚无明确共识,需要大规模、多中心的前瞻性队列研究。此外,健康生活方式也是平衡体内 ROS 和抗氧化剂水平并防止氧化应激的关键。减少吸烟、饮酒和辐射暴露,选择适当的饮食和体育锻炼,对于降低氧化应激并避免不孕不育症发生具有重要意义。

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