线粒体代谢功能对早期胚胎表观遗传组和发育的影响

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摘要 线粒体是细胞内重要的细胞器。卵母细胞成熟和早期胚胎发育期间线粒体经历动态的变化, 是支持胚胎发育的关键事件。线粒体具有多种功能, 不仅通过氧化磷酸化合成ATP, 而且参与活性氧(reactive oxygen species, ROS)的产生、调控钙离子动态平衡和信号传导。许多研究表明, 线粒体功能异常不仅诱导卵母细胞质量和附植前胚胎发育能力下降, 也能影响附植后胚胎发育, 导致个体成年后患病。越来越多的证据表明, 卵母细胞和胚胎线粒体活性及动力学通过改变代谢辅助因子的可用性而调控表观遗传修饰剂的活性, 建立表观遗传图谱的持久改变。据此提出, 附植前胚胎发育可能是一个敏感的窗口期, 线粒体参与该时期表观遗传调控可能对胚胎发育和后代的健康产生影响。基于此, 该文综述了线粒体功能及其对早期胚胎发育表观遗传组和发育能力的影响, 为通过调控线粒体功能而增强卵母细胞质量和胚胎发育能力, 以及提高家畜繁殖力提供理论依据。

关键词 线粒体功能; 代谢中间物; 表观遗传组; 胚胎发育

The Effect of Mitochondrial Function on Epigenome and Development in Preimplantation Embryos

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Abstract Mitochondria are important intracellular organelles that undergo dynamic restructuring and redistribution during oocyte maturation and preimplantation embryo development, necessary to support key developmental events. Mitochondria fulfil a wide range of functions beyond ATP synthesis, including intracellular reactive oxygen species production and the control of calcium homeostasis and signal transduction. Some studies have shown that mitochondrial dysfunction lead to not only reduce oocyte quality and embryo development, but also contribute to post-implantation failure and adult disease. A growing body of evidence indicates that oocyte and embryo mitochondrial activity and their dynamics have the capacity to establish long-lasting alterations to the epigenetic landscape by regulating availability of metabolic co-factors in modulating the activity of epigenetic modifers. It is proposed that preimplantation embryo development represents a sensitive window during which epigenetic regulation by mitochondria is likely to have signifcant effects on embryo development, and offspring health. Hence, in this paper, we review mitochondrial metabolism function and its effects on epigenome and development of preimplantation embryos, which will offer novel strategies to enhance oocyte quality and embryo development, thereby improving animal fertility.

Keywords mitochondria function; metabolic intermediate; epigenome; embryo development

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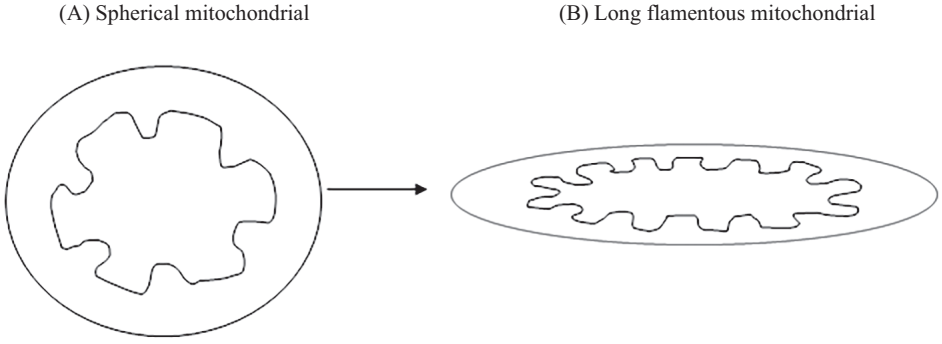
线粒体是双膜结合的细胞器, 主要通过氧化磷酸化合成ATP为细胞生命活动提供能量, 同时也参与活性氧(reactive oxygen species, ROS)的产生、钙离子动态平衡和信号传导等过程的调控。目前已明确线粒体是卵母细胞内最丰富的细胞器, 而且胚胎发育期间其形态、结构和功能经历了动态的变化, 是支持胚胎发育的重要过程[1]。许多研究表明, 线粒体功能异常不仅导致卵母细胞质量和附植前胚胎发育能力下降, 也能影响附植后胚胎发育, 甚至导致动物个体成年后患病[2-4]。越来越多的证据表明, 卵母细胞和胚胎线粒体活性及动力学通过改变代谢辅助因子的可用性而调控表观遗传修饰剂的活性, 建立表观遗传图谱的持久改变, 说明线粒体参与的代谢过程调控了附植前胚胎发育期间的表观遗传修饰, 这可能对胚胎发育和后代的健康产生深刻的影响[5-6]。鉴于此, 为了科学合理地调控线粒体功能而增强卵母细胞质量和胚胎发育能力, 本文综述了线粒体功能及其对早期胚胎发育表观遗传组和发育能力的影响。

1 线粒体的形态结构变化

目前研究已确定, 附植前胚胎发育过程中, 线粒体形态和结构经历动态的变化, 由具有密集的基质、弧形嵴的球形分化为含有横嵴的长丝状(图1)构成, 进而增加内膜表面积, 提高氧化磷酸化代谢能力, 这不仅反映了胚胎不同阶段发育代谢需求的差异, 也体现了线粒体信号转导通路的交叉联系的完善[7]。一般认为, 成熟的卵母细胞含有丰富球形的线粒体, 尽管球形被认为是线粒体不成熟的形态, 但这些线粒体具有活性, 能够产生大量的ATP支持受精后的早期胚胎发育。随着胚胎卵裂, 其线粒体形态变长、出现发育成熟的横嵴, 且具有延长和丰富嵴的线粒体数量逐渐增加。胚胎发育至囊胚阶段, 滋养层细胞线粒体为长条形, 而内细胞团细胞线粒体仍为与卵母细胞相似的球形, 这两种不同类型细胞线粒体结构的差异导致氧化磷酸化代谢活性不同, 同时反映出能量需求不同。这提示, 线粒体上述变化有利于胚胎附植, 且可能通过调节中间代谢物的可获得性而影响胚胎细胞的表观遗传组, 进而调控其分化[8-10]。近年来, 众多文献指出, 线粒体是调控胚胎干细胞谱系决定的核心因子, 而且与其相关的代谢产物逐渐被认为是细胞多能性和命运的调控者[6,11-12]。这些研究结果说明, 线粒体形态结构的变化与其代谢活性密切相关, 且在早期胚胎发育期间发挥了重要作用。

2 线粒体的代谢功能

普遍认为线粒体是细胞内的能量工厂, 通过氧化磷酸化合成ATP而为细胞代谢提供能量。电子传递链(electron transport chain, ETC)由5个复合体组成, 位于线粒体内膜, 调节还原型烟酰胺腺嘌呤二核苷酸(NADH)和琥珀酸通过ETC传递, 产生电化学梯度穿过线粒体内膜而驱动ATP合成酶产生ATP。目前研究认为, 呼吸链复合体在线粒体内膜上装配成超级复合体决定细胞呼吸活动的强弱[13]。呼吸链单个复合体被认为能够灵活地适应环境变化。线粒体内



附植前胚胎发育过程中, 线粒体形态和结构经历动态的变化, 由具有少量弧形嵴的球形(A)分化为含有丰富横嵴的长丝状(B)。
During preimplantation embryo development, mitochondria undergo dynamic changes of morphology and structure, morphing from spherical organelles containing few peripheral arched cristae (A) to long flamentous organelles with numerou transverse cristae (B).

图1 早期胚胎发育期间线粒体形态结构的变化

Fig.1 The changes of mitochondria morphology and structure during preimplantation embryo development

膜的环境与细胞质不同, 其拥有的穿梭系统调节细胞质和线粒体之间的特定代谢物水平而维持细胞代谢稳态[14]。值得注意的是, 许多文献指出, 线粒体代谢也合成脂肪酸、氨基酸和核苷酸等大分子, 进而支持细胞增殖[15-17]。这些发现说明, 线粒体作为细胞代谢的调控者具有多种功能, 超出了传统上所认为的代谢供能的调节作用。

动物细胞在各种刺激条件下, 线粒体一直被认为只是作为信号传导的效应器。然而, 随着人们对线粒体功能的探究, 其逐渐被视为抑制剂或激活剂而影响代谢物的有效性和氧化还原状态的变化, 进而参与众多代谢通路的调控[18]。线粒体除了通过氧化磷酸化产生ATP之外, 也参与调控细胞钙离子动态平衡、胁迫反应、凋亡等诸多生物过程, 甚至有研究提出线粒体参与染色体分离的调控[19]。另外, 线粒体通过氧化磷酸化代谢产生ATP的同时, 也产生ROS。目前研究认为, 线粒体产生高水平的ROS对细胞不利, 但生理浓度的ROS作为信号分子通过修饰激酶、转录因子、生长因子和代谢酶而直接调节许多生物过程[7]。这些研究结果说明, 线粒体是维持细胞代谢动态平衡的关键细胞器, 其在不同的生理和病理过程中发挥重要作用。

更为重要的是, 线粒体与细胞特化密切相关, 动物细胞系定型需要功能性线粒体适应而支持细胞分化[20]。动物细胞能量需求的变化很大程度上取决于细胞功能和活动, 因此需要调控能量产生以适应生理需求的变化, 这是维持细胞身份的基础。线粒体的代谢产物曾被认为只是细胞的代谢副产物, 然而, 近年的研究支持线粒体代谢物作为信号分子通过调控表观遗传而影响基因表达的说法, 从而驱动细胞谱系决定[12,21]。利用抗霉素和解偶联剂羰基氰化物间氯苯腙抑制线粒体的活性阻止胚胎干细胞的分化, 提示线粒体活性是干细胞分化的决定因素[22]。特别注意的是, 线粒体代谢控制乙酰辅酶A、α-酮戊二酸(α-ketoglutarate, α-KG)和NADH/NAD^+等关键的表观调控辅助因子水平, 也调控柠檬酸盐和琥珀酸盐等三羧酸代谢中间产物的水平, 这些中间产物是表观遗修饰剂的关键底物[23]。尽管目前关于胚胎干细胞的研究已强调营养调节在建立表观基因组方面的重要性, 但我们在理解调节营养和代谢中间产物对卵母细胞和附植前胚胎发育过程表观遗传的调控作用仍处于初始阶段。

3 早期胚胎的表观遗传组与胚胎发育关系

附植前胚胎经历表观遗传图谱广泛的重编程以支持胚胎基因组激活和细胞谱系形成, 是其发育过程中的重要特点。受精后, 父系基因组经历了活跃的DNA去甲基过程, 随着胚胎卵裂, 被动去甲基持续进行直到桑椹胚阶段。囊胚的形成导致细胞系的建立, 其中多能性内细胞团细胞相对于滋养层细胞表现出较高的从头甲基化水平。除了DNA甲基化, 染色质重塑和组蛋白修饰也在胚胎表观遗传图谱重新编程方面挥关键作用。然而, 相对于DNA甲基化, 关于组蛋白乙酰化和甲基化动力学主要局限于组蛋白乙酰化酶、甲基转移酶和去甲基酶表达方面的研究[24]。尽管如此, 目前已确定早期胚胎正常发育依赖DNA甲基化、组蛋白乙酰化和甲基化的协调变化, 这些变化支持了胚胎特定基因的激活和沉默[25-27]。

研究表明, 早期胚胎表观遗传图谱异常与胚胎发育的微环境有关, 将导致胚胎发育异常[28-29]。另有研究发现, 兔子胚胎体外培养期间促进DNA甲基化[30], 体内获取的牛胚胎体外培养期间也促进DNA的甲基化[31], 而且小鼠胚胎体外培养期间显著降低组蛋白第三亚基四号赖氨酸的三甲基化(H3K4me3)[32]。近年研究表明, 胚胎体外培养提高组蛋白第三亚基九号赖氨酸的乙酰化(H3K9ac)水平, 是导致表观遗传图谱异常的一个重要标志[33]。更重要的是, 研究发现, 抑制胚胎组蛋白去乙酰化酶活性降低或抑制囊胚发育, 而且干扰早期胚胎表观遗传组重新编程, 不仅阻碍胚胎附植, 且导致附植后胚胎蜕膜化异常[34-37]。这些研究说明, 早期胚胎表观遗传组的变化对其所处的营养环境十分敏感, 早期胚胎的表观遗传图谱的异常将影响胚胎发育。

4 线粒体代谢相关产物与早期胚胎表观遗传组

4.1 NAD^+参与调控早期胚胎的组蛋白乙酰化

研究已确定, 调节沉积和去除DNA和组蛋白标记的表观遗传修饰活性依赖特殊线粒体代谢物的可获得性[38]。组蛋白的乙酰化受组蛋白乙酰化和去乙酰化酶调控。乙酰转移酶乙酰化组蛋白需要线粒体代谢中间产物乙酰辅酶A, 其可获得性决定了组蛋白乙酰化水平, 从而影响基因表达。尽管细胞质中柠檬酸、脂肪酸和酮体氧化和氨基酸的分解代谢可产生乙酰辅酶A, 但提高糖酵解速率能够驱动线粒

体合成乙酰辅酶A[39]。近年研究表明, 葡萄糖通过糖酵解代谢上调胚胎干细胞乙酰化水平, 进而促进组蛋白的乙酰化, 而且使用乙酰辅酶A前体醋酸盐处理胚胎干细胞提高乙酰化水平, 阻止组蛋白去乙酰化, 降低干细胞的分化; 相反, 抑制葡萄糖产生乙酰辅酶A导致胚胎干细胞失去全能性[10]。这些研究暗示, 葡萄糖的利用变化不仅可以显著影响细胞代谢物水平, 而且通过调控乙酰辅酶A水平而改变组蛋白乙酰化, 从而调节胚胎干细胞分化, 因此利用葡糖糖能力被认为是胚胎活力的一个重要标志。葡萄糖摄取的改变可以显著影响乙酰辅酶A的可用性, 这对理解附植前胚胎发育过程中乙酰辅酶A水平改变的影响十分重要。

组蛋白去乙酰化部分介导基因转录的抑制。除了依赖锌作为共激活因子的I和II型组蛋白去乙酰化酶之外, 沉默信息调节子(silent information regulator, SIRT)家族成员以NAD^+作为其激活因子的去乙酰化酶。研究认为, NAD^+可获得性调控SIRT活性, SIRT被激活后能使参与细胞代谢、线粒体生物合成、细胞存活、DNA修复、分化以及活性氧产生的蛋白去乙酰化[40]。值得注意的是, SIRT活性变化能够影响胚胎发育。研究发现, 使用依妥那胺或西丁醇抑制SIRT活性显著降低了猪和小鼠的囊胚发育能力, 这可能与调控组蛋白乙酰化水平有关[41-42]。因此, 研究SIRT对附植前胚胎表观遗传组的调控作用及NAD^+调控SIRT活性十分重要。研究认为, NAD^+可由色氨酸从头合成或通过烟酰胺和维生素B3经补救合成代谢途径循环利用[43-44]。为了确保细胞持续糖酵解, NAD^+也可通过丙酮酸至乳酸转化循环利用, 促进胚胎发育[38]。NADH还原当量也经由苹果酸–天冬氨酸穿梭维持NAD^+:NADH比例而在线粒体内膜实现穿梭[45]。苹果酸–天冬氨酸穿梭活性是维持胚胎发育期间氧化代谢的必需过程, 抑制此过程损害了胚胎代谢和ATP的合成, 降低了囊胚发育、胎盘和胎儿的生长[46-47]。然而上述变化是否影响胎儿的表观遗传组尚不确定。但这些研究结果强调, NAD^+在胚胎发育期间表观重新编程方面发挥重要作用, 需要协调细胞质和线粒体之间的代谢而维持线粒体功能, 保证胚胎正常发育。

4.2 蛋氨酸代谢参与调控早期胚胎DNA和组蛋白甲基化

DNA甲基转移酶激活后将催化甲基基团添加在胞嘧啶鸟嘌呤二核苷酸(CPG)内的5′胞嘧残基。组蛋白甲基转移酶催化转移单甲基化(me)、二甲基化(me2)及三甲基化(me3)基团至组蛋白赖氨酸和精氨酸残基上, 此过程依赖于中间代谢产物的可获得性。普遍认为, 蛋氨酸是合成甲基供体S-腺苷甲硫氨酸(S-adenosyl methionine, SAM)的前体物质。早期研究认为, 蛋氨酸在甲硫氨酸腺苷转移酶催化下转化为SAM, 是细胞甲基化反应的辅助因子[48]。近年研究发现, 蛋氨酸是小鼠和牛囊胚形成的必需因子, 胚胎培养液缺失蛋氨酸降低囊胚形成和H3K4me3水平, 胚胎基因组激活期间蛋氨酸拮抗剂损害囊胚发育、降低胚胎细胞数, 同时导致Nanog和Tead4蛋白H3K9me3水平下降[49-50]。这些研究结果暗示, SAM是胚胎基因组激活期间必需的代谢物质。

特别重要的是, 抑制甲硫氨酸腺苷转移酶2A合成SAM可显著降低小鼠和牛胚胎的发育能力和细胞数、促进胚胎细胞凋亡[50-51]。相反, 牛胚胎体外培养期间, 添加SAM增强孵化囊胚比例, 显著改变全基因组水平的DNA甲基化图谱[52]。同样, 人类胚胎干细胞培养期间, 培养液缺少蛋氨酸降低SAM水平, 导致胚胎干细胞H3K4me3和全基因组DNA甲基化水平下降, 但是补充SAM可缓解上述不利影响, 维持随后驱动人类胚胎干细胞分化的正常甲基化水平[9]。这些研究数据说明, 蛋氨酸不仅对维持胚胎干细胞多能性表观基因组很重要, 而且在改变干细胞谱系专向分化时机方面发挥重要作用。另外, 母体补充蛋氨酸的研究发现, 改变牛囊胚基因表达, 暗示蛋氨酸供应水平的细微变化甚至能够影响胚胎发育[53]。

叶酸和维生素B12是叶酸和蛋氨酸循环的交叉代谢物质, 这些代谢物缺乏时也显著影响胚胎发育、出生后生长发育和基因组甲基化水平, 而且, 叶酸循环的代谢物苏氨酸调控小鼠胚胎干细胞的SAM水平[8,54]。引起人们广泛关注的研究发现, 细胞质叶酸循环与线粒体甲酸酯可用性存在交叉作用, 而细胞线粒体NADH氧化和三羧酸循环活性与蛋氨酸代谢密切相关, 这些研究强调线粒体代谢活性与胚胎甲基化密切相关[55]。因此, 线粒体一碳化合物代谢整合营养、氨基酸代谢和线粒体活性, 导致代谢途径流量变化而深刻影响表观基因组。

4.3 α-酮戊二酸参与调控早期胚胎的组蛋白乙酰化

α-KG是葡萄糖和谷氨酰胺经由线粒体三羧

酸循环代谢的中间产物。研究发现, α-KG能够调控双加氧酶TET(ten-eleven translocation)和组蛋白去甲基化酶JMJ的活性[56-57]。TET蛋白家族成员包括TET1、TET2和TET3, 是α-KG和Fe^{2+}依赖的双加氧酶, 具有将5-甲基胞嘧啶(5-methylcytosine, 5mC)氧化为5-羟甲基胞嘧啶(5-hydroxymethylcytosine, 5hmC)的能力。研究认为, 受精卵的原核期, TET3介导激活了父系基因组的去甲基化直到8细胞胚胎阶段[58]。猪胚胎基因组激活期间, TET3调控了多能性基因*Nanog*表达, 说明其参与了胚胎干细胞谱系专向分化[59]。小鼠早期胚胎发育期间和猪4细胞胚胎期之后表达TET1, 而且囊胚内细胞团细胞相对于滋养层细胞具有丰富的TET1[59-60], 这些研究支持其参与胚胎干细胞谱系专向分化的结论, 同时, TET1在囊胚细胞上的差异表达也反映了内细胞团和滋养层细胞之间代谢存在差异。葡萄糖和谷氨酰胺代谢产物α-KG调节小鼠胚胎干细胞组蛋白和TET依赖的DNA去甲基化, 揭示糖酵解活性支持细胞去甲基化, 适宜的吸收葡萄糖可能是建立正确的甲基化/去甲基化动态平衡必需的过程, 这也可以部分解释吸收葡萄糖与胚胎活力存在相关性[61]。确实, 研究发现, 小鼠体外培养的胚胎相对于体内培养的胚胎提高了DNA甲基化水平, 说明体外培养导致去甲基化酶调控DNA去甲基化的功能异常[62]。因此, 研究调节α-KG产生是否能够影响胚胎发育和DNA甲基水平变化十分重要。

另外, JMJ(jumonji)是依赖α-KG和Fe^{2+}的组蛋白去甲基化酶, 通过去除组蛋白赖氨酸残基的甲基基团而实现组蛋白去甲基化。普遍认为, JMJ适当地调控组蛋白去甲基化是胚胎正常发育必需的过程。研究发现, *JMJD3*是2细胞阶段后胚胎发育必需的H3K27me3去甲基化因子, 其敲除后导致小鼠和牛囊胚发育能力和细胞数下降[63-64]。研究发现, 小鼠胚胎干细胞α-KG可利用性与JMJD3活性密切相关, 这说明, 线粒体代谢中间产物α-KG可能通过调控JMJ活性而影响组蛋白的甲基化[61]。

综上所述, 线粒体代谢活性在早期胚胎表观遗传调控方面发挥重要作用, 是连接环境刺激与胚胎表观基因组变化间的基本整合因子。干扰代谢途径影响调节表观遗传修饰剂所需的辅助因子产生和可获得性, 导致表观重编程干细胞谱系专向分化期间细胞核表观图谱的可遗传的变化。因此, 干扰附植前胚胎周围营养环境可能通过改变影响表观基因组重编程的新陈代谢来调节后代的健康和生存能力。早期胚胎发育期间, 线粒体的动态特性不仅产生ATP, 而且能够阶段特异性地促进和调节早期胚胎表观遗传修饰的沉积和去除。

5 线粒体代谢功能与早期胚胎发育

早期研究发现, 卵母细胞和早期胚胎线粒体必须产生足够数量ATP才能支持支持早期胚胎发育[65]。随后, 众多研究表明, 哺乳动物附植前胚胎发育期间经历显著的代谢变化, 而且卵母细胞和胚胎之间代谢通路活性和ATP含量存在显著差异[66-68]。目前已明确, 卵丘颗粒细胞通过糖酵解产生丙酮酸提供给卵母细胞, 卵母细胞通过氧化磷酸化代谢丙酮酸产生能量[69]。早期胚胎在基因组激活之前与卵母细胞相似, 可以利用丙酮酸、乳酸和氨基酸进行低水平的氧化代谢而支持胚胎发育, 随着胚胎基因组激活和胚泡腔的形成, 囊胚阶段的胚胎显示高水平糖酵解和耗氧量[69]。因此, 早期胚胎发育期间能量需求的变化需要动态协同调控代谢而支持随后的发育。

研究发现, 卵母细胞含有高水平的ATP而展示了较高的受精率和囊胚发育能力, 相反卵母细胞缺乏ATP导致较差的卵母细胞质量和胚胎发育能力[70]。另有研究发现, 干扰线粒体活性影响胚胎发育、附植潜力及胎儿胎盘的生长, 这进一步说明, 线粒体产生ATP支持胚胎发育[71-72]。因此, 为了增强线粒体功能, 许多学者使用辅酶Q10、白藜芦醇、*L*-肉碱以及α-硫辛酸等调控线粒体代谢的功能物质处理卵母细胞和早期胚胎, 发现提高了卵母细胞质量和胚胎发育能力[73-76]。这些研究结果充分证明, 线粒体活性是卵母细胞成熟和早期胚胎发育潜能的标志, 但这仅从线粒体产生ATP能力的角度探讨其与胚胎发育能力的关系, 而没有考虑线粒体作为重要细胞器在调控细胞其他方面动态平衡的作用。

值得注意的是, 研究已确定老化或胁迫因子诱导线粒体功能异常, 不仅影响卵母细胞或胚胎线粒体产生ATP能力, 而且导致调控表观图谱的代谢中间产物供应能力下降。确实, 卵母细胞老化后5-甲基胞嘧啶、DNMT表达、H4K12/H4K16乙酰化以及组蛋白表达水平下降[77]。另有研究发现, 人受精卵线粒体功能异常后降低NADH/NADPH比例, 但这

种变化是否会调控NAD^+依赖的去乙酰化酶SIRT的活性尚不确定[78]。这些结果提示, 线粒体功能相关的代谢活性改变介导卵母细胞和/或早期胚胎的表观图谱变化, 从而影响随后的胚胎发育能力, 但线粒体功能异常相关的代谢干扰而对早期胚胎乙酰辅酶A、α-KG等代谢物水平的影响以及代谢物水平变化调控表观图谱而对胚胎发育和后代的健康作用尚不十分清楚, 仍需深入调查。

6 结语

综上所述, 线粒体不仅通过氧化代谢产生ATP为卵母细胞和胚胎提供能量, 而且其作为动态变化的细胞器与细胞核和其他细胞相互作用产生中间代谢产物参与信号传导, 影响染色体分离、胚胎干细胞谱系专向分化。线粒体功能异常将降低胚胎发育能力。由于线粒体相关代谢通路代谢流量影响调控表观遗传修饰的辅助因子可获得性, 线粒体逐渐被认为是整合代谢和信号传导的细胞器传感器, 也可以部分解释代谢异常所引起可遗传性表观变化而导致个体发育能力较差的原因。基于以上分析, 我们认为, 通过科学合理的方法调控线粒体功能可能缓解代谢异常而导致胚胎表观遗传组的变化, 增强卵母细胞质量和胚胎发育能力, 有利于提高家畜的繁殖性和人类辅助生殖技术的应用效率。

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