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钙离子在哺乳动物卵母细胞发育关键时期的作用

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摘 要 Ca^{2+}是广泛存在的信号分子,钙信号转导对多种细胞生理生化活动有重要的调控作用,越来越多的证据表明钙信号参与卵母细胞减数分裂。钙稳态是细胞内Ca^{2+}信号在时间和空间上的动态平衡,对卵母细胞钙稳态的研究是目前重要的研究热点之一。本文综述了钙稳态及钙离子对卵母细胞成熟和发育过程中的双线期阻滞恢复、MII期阻滞恢复和细胞凋亡的作用,发现某些哺乳动物双线期及MII期适当增加胞内钙离子水平可以使卵母细胞退出阻滞,而超出生理水平的胞内钙则会诱导细胞凋亡,并通过探究钙离子在卵母细胞减数分裂恢复和调控凋亡过程中的作用机制,为辅助生殖技术的进一步研究提供依据。

关键词 卵母细胞;Ca^{2+};减数分裂恢复;钙稳态
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Role of calcium ion in the critical development periods of mammalian oocytes

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Abstract Ca^{2+} is a ubiquitous signaling molecule. Calcium signal transduction plays an important regulatory role in a variety of physiological and biochemical activities of cells. More and more evidence shows that Calcium signal is involved in oocytes meiosis. Calcium homeostasis is the dynamic balance of intracellular Ca^{2+} signals in both time and space. The study of calcium homeostasis in oocytes is one of the important research hotspots. This article reviews the effects of calcium homeostasis and calcium on the meiotic resumption from diplotene arrest, meiotic resumption from MII arrest and apoptosis. Studies have found that in certain mammals, oocyte exit the block from both the diplotene and MII phases by appropriately increasing intracellular calcium levels, however, intracellular calcium that exceeds the physiological level will induce apoptosis. To explore the mechanism of calcium ions in meiotic resumption and apoptosis in oocytes is beneficial to the further study of assisted reproductive technology.

Keywords oocyte; Ca^{2+}; meiotic resumption; calcium homeostatic

哺乳动物卵母细胞是形成胚胎的基础,而双线期阻滞恢复和MII阻滞恢复是卵母细胞发育的关键,目前对卵母细胞的研究一直是生殖生物学研究的热点和重点。Ca^{2+}是广泛存在的信号分子,钙信号转导对多种细胞生理生化活动有重要的调控作用,越来越多的证据表明Ca^{2+}信号参与卵母细胞减数分裂。1981年,Paleos和Powers[1]发现卵母细胞在无钙培养基中不能排出第一极体。1992年,Carroll和Swann[2]第一次在小鼠卵母细胞体外成熟过程中发现存在自发Ca^{2+}波动,且这种波动与胞质成熟有关。同年,Tombes等[3]发现小鼠卵母细胞体外成熟并不依赖于胞内钙的变化。1993年,Homa等[4]通过向卵母细胞显微注射Ca^{2+}激动剂和抑制剂发现胞内自由Ca^{2+}是固有信号的信使,参与卵母细胞减数分裂过程的调控。1995年,Homa[5]提出在卵母细胞有丝分裂和减数分裂过程

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都有钙信号参与调控，LH 诱导减数分裂恢复引起钙动员，钙离子抑制腺苷酸环化酶，打破减数分裂阻滞，钙离子通过 CAMKII 调控细胞周期蛋白活性。细胞膜上及细胞内不同部位分布着各种 Ca^{2+} 通道和受体，在 Ca^{2+} 转运过程中起着各自不同的作用，由此诱发的胞浆 Ca^{2+} 瞬变参与调节胞内多种生理生化活动。通过调控和串联 Ca^{2+} 释放，内流，外排，隔离及缓冲机制，最终调控 Ca^{2+} 信号在时间和空间上的动态平衡被称之为钙稳态[6]。1996 年，Duesbery 和 Masui[7]对非洲爪蟾的研究中一致认为 Ca^{2+} 增加能有效诱导卵母细胞成熟，注射 Ca^{2+} 缓冲液会阻断成熟。2005 年，Mehlmann[8]也提出钙离子螯合剂可以延迟减数分裂的发生。2008 年，Sun 等[9]发现在非洲爪蟾卵母细胞成熟过程中胞质钙离子对进入减数分裂和 GVBD 发生可有可无，但钙稳态对 MI 的完成和第一极体的排出是必需的。2011 年，Liang 等[10]发现牛卵母细胞成熟过程中胞内钙离子会经历逐渐升高，MI 期达到最高，然后再降低，最终 MII 时期 Ca^{2+} 水平略高于 GV 期，且从 GV 期到 MII 期 Ca^{2+} 有从皮质区向中间扩散的变化。2017 年，Courjaret 等[11]发现 IP_3R 作为细胞内多种信号传导通路的整合分子介导了细胞内钙库释放 Ca^{2+}。

1　卵母细胞中的钙稳态

钙稳态对维持卵母细胞成熟期间细胞形态和胞内生化过程之间的同步性非常重要。游离 Ca^{2+} 浓度（$[Ca^{2+}]$）根据在细胞内的位置不同而有所差异。胞质内 $[Ca^{2+}]$ 处于静息状态的时维持在大约 10^{-7} mol/L，比细胞外环境的大约 10^{-3} mol/L 的水平低了 10^4 倍，细胞内的核内 $[Ca^{2+}]$ 及线粒体内 $[Ca^{2+}]$ 与 $[Ca^{2+}]_c$ 水平相近，然而，被称为钙库的细胞内的其他细胞器，能够储存 Ca^{2+}，并维持比 $[Ca^{2+}]_c$ 更高的 $[Ca^{2+}]$（1～5 10^{-4} mol/L）[12]。卵母细胞内主要的钙库是内质网，但在肌肉细胞里面主要是肌浆网。

几乎细胞功能的每一个方面都由 Ca^{2+} 调控，包括分泌、基因表达、肌肉收缩和代谢等，因此，静息状态下低 $[Ca^{2+}]_i$ 和钙信号必须受到严格的调控，任何不受控制的 $[Ca^{2+}]_i$ 升高都会导致细胞损伤或细胞死亡[13-14]。一些细胞刺激，例如：膜去极化、胞外信号分子或者胞内信使能促使胞内自由钙离子从 100 nmol/L 增加到 1 mmol/L，甚至更高，这种 Ca^{2+} 增加可能源于胞外 Ca^{2+} 经质膜（Plasma membrane，PM）钙通道内流也可能是由胞内钙库释放引起，胞内钙库钙释放大多是通过 IP_3R 和 RyR 通道。在 $[Ca^{2+}]_i$ 升高后，肌浆内质网 Ca^{2+}-ATP 酶（SERCA）将胞质增加的钙离子存进内质网钙库内，在较小程度上由线粒体 Ca^{2+} 单向转运体（mtCU）转入线粒体，线粒体作为低亲和力、高效率的缓冲室，可以吸收掉大量 Ca^{2+}，再随着时间推移慢慢释放出来[15-16]。所有这些蛋白对 Ca^{2+} 的感知都是被 Ca^{2+} 激活的，因此，任何 $[Ca^{2+}]_i$ 升高都能刺激细胞质 Ca^{2+} 的移除，从而导致 $[Ca^{2+}]_i$ 的稳态调控[13]。静息态细胞的低 $[Ca^{2+}]_i$ 主要通过 Na^+/Ca^{2+} 交换器（Na^+/Ca^{2+} exchanger，NCX）或者质膜 Ca^{2+} 运输 ATP 酶（Plasma membrane Ca^{2+} transport ATPase，PMCA）维持，NCX 是高能力、低亲和力运输器，当胞质内 Ca^{2+} 浓度达到毫摩时，运用 Na^+ 梯度将大量 Ca^{2+} 排出[17]，而 PMCA 对 Ca^{2+} 有很高亲和性，将 Ca^{2+} 水平微调到静息态。肌浆内质网 Ca^{2+}-ATP 酶（Sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase，SERCA）将 Ca^{2+} 收入内质网腔[18]。

各类型的细胞都存在独特的钙通道和钙泵组合，以适应生理需求。钙库 Ca^{2+} 释放、胞外钙内流、胞内钙外排、胞内缓冲机制间的校准和互作最终调控 Ca^{2+} 信号的时空动态。大多数哺乳动物的生殖细胞，GV 期和受精前的 MII 期卵母细胞就需要维持低 $[Ca^{2+}]_i$ 水平，而 GVBD 的发生、第一极体的排出和受精卵母细胞的活化则需要很高 $[Ca^{2+}]_i$ 水平，所有这些过程的完成就需要卵母细胞内的钙稳态机制密切调控。

2　Ca^{2+} 在双线期卵母细胞从阻滞中恢复的作用

在卵母细胞减数分裂从双线期阻滞恢复过程中，Ca^{2+} 是重要的信号分子发挥核心作用[19]。双线期阻滞的卵母细胞叫做终线期卵母细胞，是根据原始卵泡中卵母细胞胞质和核的形态而鉴定。哺乳动物卵母细胞双线期阻滞的时长，依物种而异，有的物种可以仅几个月，有的长达几年[8, 20]。

卵母细胞正常成熟时，适当增加 $[Ca^{2+}]_i$ 水平会引发一定范围内活性氧（Reactive oxygen

species，ROS）产生，介导减数分裂自发从双线期阻滞恢复[21-23]。减数分裂从双线期阻滞恢复期间，胞质内 cAMP 水平降低以及钙水平增加诱导 ROS 产生，适当增加的 $[Ca^{2+}]_i$ 以及 ROS 能使成熟促进因子（Maturation promoting factor，MPF）去稳定化[6, 23-24]，$[Ca^{2+}]_i$ 和 ROS 水平增加引起钙-钙调蛋白依赖蛋白激酶 II（Calcium-calmodulin-dependent protein kinase II，CaMKII）的活化[25]，活化的 CaMKII 激活酪氨酸激酶 Wee1，Wee1 通过诱导 MPF 的催化亚基 Cdk1 的 Thr14/Tyr15 磷酸化，调节亚基 cyclin B1 降解，去稳定 MPF[26-27]。因此，适当增加的 Ca^{2+} 及 ROS、短暂降低 cAMP 等信号分子使 MPF 去稳定或者活化 Cdk1，最终导致哺乳动物卵母细胞减数分裂从双线期阻滞恢复。

在体外成熟培养时，胞外钙离子可诱导小鼠、大鼠、牛、猪卵母细胞从双线期阻滞中恢复[21, 28-30]。钙离子载体和离子霉素等药物可诱导钙从胞内钙库释放，引起小鼠、大鼠、牛、猪、人卵母细胞减数分裂从双线期阻滞中恢复[26, 31-33]。钙通道阻断剂可抑制小鼠、大鼠、猪、牛卵母细胞自发的减数分裂恢复[27, 34]。以上研究表明双线期适当增加 $[Ca^{2+}]_i$ 水平可以使卵母细胞减数分裂从其阻滞中恢复。

3 Ca^{2+} 在 MII 期卵母细胞从阻滞中恢复的作用

MII 期卵母细胞胞内钙水平的适当增加可以使其从阻滞中恢复减数分裂。排卵后的哺乳动物卵母细胞在生理上被阻滞在 MII 阶段，这些排出的卵母细胞在输卵管的壶腹部等待与精子受精[35]。精子进入卵母细胞诱导其胞内钙库释放钙离子，引起卵子活化（Oocyte activation，OA），减数分裂恢复[36]。精子诱导 OA 期间钙振荡增加，直到原核形成，OA 以皮层颗粒胞外分泌、MII 阻滞恢复、排出第二极体、原核形成等一系列的形态变化及分子事件为特征[19, 37-38]。自发卵母细胞活化（Spontaneous oocyte activation，SOA）具备除了原核形成和排出第二极体外 OA 的所有特征[39]，但是这种卵母细胞自发活化，退出 MII 阻滞，会降低卵母细胞质量并限制了可用于辅助生殖技术的哺乳动物卵母细胞数量。

与卵母细胞从双线期阻滞恢复减数分裂的机制相似，$[Ca^{2+}]_i$ 水平增加可引起 CaMKII 活化并产生 ROS，CaMKII 继而激活 Wee1，活化的 Wee1 诱导 cyclin B1 降解及 Cdk1 的 Thr14/Tyr15 磷酸化，从而使 MPF 不稳定，最终导致卵母细胞自发退出 MII 阻滞，这些都在与大鼠和小鼠卵母细胞相关的研究中得到验证[25, 27, 40]。大鼠卵母细胞 SOA 时 $[Ca^{2+}]_i$ 增加 1.4 倍，OA 时 $[Ca^{2+}]_i$ 增加 2.5 倍[39, 41-42]。在 MII 期，异常高浓度的 $[Ca^{2+}]_i$ 会影响卵母细胞的发育，而在培养基中添加胞内钙离子螯合剂 BAPTA-AM 降低 $[Ca^{2+}]_i$ 浓度，可提高成熟率，增加卵母细胞的数量[43]。

4 Ca^{2+} 可诱导卵母细胞凋亡

$[Ca^{2+}]_i$ 水平的动态变化调控卵母细胞生理活动，研究发现持续超出正常生理范围的 $[Ca^{2+}]_i$ 会诱导哺乳动物卵母细胞的凋亡[44]。当向培养基添加高浓度钙离子载体时，会导致大鼠、猪、牛卵母细胞细胞周期受阻，并发生凋亡[45-48]。

研究发现，在体外培养条件下老化的卵母细胞容易发生损伤。究其原因，持续高水平的胞内游离钙离子诱导了 CaMKII 的活化并产生足以引起氧化应激（Oxidative stress，OS）的 ROS。在老化的卵母细胞中，持续 MPF 降低或者 OS 增加都会引起 Bax 过表达和 Bcl-2 的低表达，引起线粒体膜上 Bax/Bcl-2 比率改变，膜电势随之改变，促使卵母细胞内细胞色素 C 增加，进而激活细胞内上、下游的凋亡蛋白酶，诱导细胞凋亡[46, 49-52]。2016 年，Zhu 等[53]研究发现高水平的 $[Ca^{2+}]$ 还可诱发 Fas 配体介导的凋亡。越来越多的证据表明，持续高浓度的 $[Ca^{2+}]_i$ 会诱导体外培养的老化卵母细胞 CaMKII 活化，并产生足够导致氧应激的 ROS，CaMKII 激活 Wee1 诱导 cyclin B1 降解，Cdk1 的 Thr14/Tyr15 磷酸化，导致 MPF 不稳定，持续低水平的 MPF 则会诱导体外培养的卵母细胞发生凋亡[54, 55]。

5 结 论

综上，卵母细胞钙稳态对哺乳动物的生殖生理至关重要。卵母细胞内钙离子调控卵母细胞双线期和 MII 期阻滞恢复，并决定卵母细胞的凋亡。了解并探明钙离子在卵母细胞减数分裂恢复中的作用机制和在卵母细胞凋亡中的作用机制，将有利于补充和完善卵母细胞成熟理论，同时对探究部分不孕不育症的发病机理具有重要的理论意义，同时有利于在生

产实践中建立高效卵丘卵母细胞复合体体外成熟培养体系，提高家畜卵母细胞体外成熟质量和受精率。

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