

# 体细胞克隆猪发育异常研究进展

敖政<sup>1</sup>, 陈祥<sup>1</sup>, 吴珍芳<sup>2</sup>, 李紫聪<sup>2</sup>

1. 贵州大学动物科学学院, 高原山地动物遗传育种与繁殖教育部重点实验室, 贵阳 550025

2. 华南农业大学动物科学学院, 国家生猪种业工程研究中心, 广州 510642

**摘要:** 克隆又称体细胞核移植(somatic cell nuclear transfer, SCNT), 是一种将已分化的细胞重编程恢复全能性而生产与供体细胞基因型完全相同后代的无性繁殖技术。猪的克隆技术具有重要的应用价值, 包括扩繁优良种猪、制备基因修饰猪、保护珍贵和濒危猪种以及研究猪体细胞重编程机制。然而, 克隆猪存在出生率和初生重低以及死胎率、新生儿死亡率和畸形率高等问题, 这些都严重影响了克隆猪的应用前景。供体核的表观重编程错误被认为是克隆效率低和胚胎发育异常的主要原因, 但是目前大多数研究通过修正表观重编程错误并没有大幅度提高克隆猪的出生率与健康率。本文综述了克隆猪的异常表型、发育异常的原因以及提高猪克隆效率的有效方法, 以期为提高克隆猪的成活率提供参考。

**关键词:** 体细胞核移植; 克隆猪; 表观重编程; 发育异常

## Progress on abnormal development of cloned pigs generated by somatic cell transfer nuclear

Zheng Ao<sup>1</sup>, Xiang Chen<sup>1</sup>, Zhenfang Wu<sup>2</sup>, Zicong Li<sup>2</sup>

1. Key Laboratory of Animal Genetics, Breeding and Reproduction in the Plateau Mountainous Region, Ministry of Education, College of Animal Science, Guizhou University, Guiyang 550025, China

2. National Engineering Research Center for Swine Breeding Industry, College of Animal Science, South China Agricultural University, Guangzhou 510642, China

**Abstract:** Cloning, also known as somatic cell nuclear transfer (SCNT), is an asexual reproduction technique that reprograms differentiated cells to the totipotent state, and generates offspring with a genotype identical to the donor cells. Pig cloning technique holds great promise for propagating excellent breeding boars, generating genetically modified pigs, protecting rare and endangered pigs and studying the mechanisms of somatic cell nucleus reprogramming. However, cloned pigs suffer from various developmental defects, including low birth rate, low birth weight, and high stillbirth occurrence, neonatal mortality and congenital malformations, which severely hamper their applications. Errors in epigenetic reprogramming of donor nucleus are considered as the main causes of low cloning efficiency and abnormal embryonic develop-

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作者简介: 敖政, 博士, 讲师, 研究方向: 动物遗传育种与繁殖。E-mail: zheng780911@163.com

通讯作者: 敖政。

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ment in cloned embryos and animals. However, most studies to correct the errors in epigenetic reprogramming of cloned pig embryos have not substantially improved the birth and survival rates of cloned pigs. In this review, we summarize the abnormal phenotypes, causes of abnormal development of cloned pigs and effective methods for improving pig cloning efficiency, thereby providing a reference for the future research to improve the development and survival rates of cloned pig embryos and cloned pigs.

**Keywords:** SCNT; cloned pig; epigenetic reprogramming; abnormal development

体细胞核移植(somatic cell transfer nuclear, SCNT)是将已分化的细胞重编程恢复全能型而生产与供体细胞基因型完全相同后代的一种无性繁殖技术<sup>[1]</sup>。从 1996 年克隆羊“多莉”诞生至今,多种哺乳动物的体细胞克隆都相继取得成功,特别是 2018 年初,中国科学家宣布成功获得了存活的体细胞克隆猴(*Macaca fascicularis*),这是体细胞克隆史上的一次重大突破<sup>[2]</sup>。猪(*Sus scrofa*) SCNT 技术在农业、生物医学和基础研究领域具有重要的应用价值,包括扩繁优良种猪、制备基因修饰猪、保护珍贵和濒危猪种以及研究猪体细胞重编程机制<sup>[3,4]</sup>。然而,如表 1 所示,克隆猪的出生率低(只有 1%左右)、死胎率高(17%~32.8%),且存在新生期死亡率高(48.0%~74.5%)、畸形率高(29.5%~60.0%)和初生重低等问题<sup>[5~19]</sup>,这些都严重影响了克隆猪的应用前景。供体核的表观重编程错误被认为是克隆效率低和胚胎发育异常的主要原因,但是目前大多数研究通过修

正表观重编程错误并没有大幅度提高克隆猪的出生率 and 健康率。因此,本文综述了克隆猪的异常表型、发育异常的原因以及提高猪克隆效率的有效方法,以期为提高克隆猪的成活率提供参考。

## 1 克隆猪发育异常

### 1.1 异常表型

Schmidt 等<sup>[18]</sup>对 815 头克隆及转基因猪从出生至断奶进行了跟踪记录,结果发现新生克隆猪的死亡率为 48%,畸形率为 29.5%,尸检结果显示死亡克隆猪的主要生理缺陷分布在消化系统、循环系统、繁殖系统和骨骼肌系统,且存在诸多未发现的细微生理缺陷,这些生理缺陷严重影响机体正常的生理活动,是造成新生克隆猪死亡的直接或间接原因。因此,目前很难确定克隆猪死亡的具体机制,可能

表 1 代孕母猪怀孕率及克隆效率

Table 1 Pregnancy rate of recipient sows and cloning efficiency

代孕母猪数	移植胚胎数	怀孕率(%)	出生总仔数	活仔数	克隆效率(%)	参考文献
24	5288	54.2	58	46	1.10	[5]
66	11,911	21.2	40	—	0.34	[6]
8	904	75	19	15	2.10	[7]
265	62,090	21.5	65	51	0.10	[8]
11	2884	72.7	12	10	0.42	[9]
193	18,649	56.5	318	243	1.71	[10]
328	92,005	57.6	488	—	0.53	[11]
4	1342	75	15	5	1.12	[12]
656	228,230	62	1070	810	0.47	[13]
79	13,620	63.3	177	119	1.30	[14]
4	1187	50	6	6	0.51	[15]
5	530	100	11	—	2.07	[16]

— 表示参考文献无相应数据;克隆效率等于出生总仔数与移植胚胎数的比值。

是一种或多种缺陷引发的结果。此外,很多新生克隆猪是无征兆的突然死亡,早期研究发现脑膜炎和血运障碍可能是仔猪突然死亡的主要原因<sup>[6]</sup>。克隆仔猪因屈肌腱和肘关节骨骼发育不良而导致仔猪站立失败,且新生克隆猪会表现出不佳的哺乳反射,因此需要人工及时喂养充足的初乳以增加仔猪的存活率<sup>[6]</sup>。

克隆新生儿呼吸窘迫是克隆动物围产期死亡的原因之一,其病理特征包括不完整的肺扩张、肺泡塌陷、肺泡壁增厚、肺内表面活性剂稳态紊乱和透明膜异常,表明克隆新生儿的肺泡功能不全而造成呼吸窘迫<sup>[20,21]</sup>。克隆猪具有如肺动脉移位、肺畸形和肺发育不全等先天缺陷,这些都可能是仔猪围产期死亡的原因<sup>[18]</sup>。Park 等<sup>[22]</sup>比较分析了出生后死亡及存活一个月克隆猪与同龄人工授精(artificial insemination, AI)猪肺脏的基因表达模式,在出生后死亡克隆猪中鉴定出 121 个差异表达基因(differentially expressed genes, DEGs),这些 DEGs 可能与克隆猪肺表面活性剂稳态失调和糖尿病性肾病等病理症状密切相关;在存活克隆猪中鉴定到 154 个 DEGs,基因功能富集分析发现这些 DEGs 与肺泡发育延迟和 MAPK 信号通路下调有关,表明存活克隆猪仍然有器官功能异常的风险<sup>[23]</sup>。此外,部分克隆猪患有巨舌,这严重影响到仔猪的摄乳和呼吸,先天和环境因素导致这类仔猪几乎不能存活<sup>[6,18]</sup>。最近,本课题组研究发现,嘌呤代谢异常可能是克隆猪肾脏发生病理变化以及新生期死亡的重要原因<sup>[24]</sup>。

## 1.2 宫内发育不良

克隆胚胎的形态和功能异常是克隆猪出生率低的重要原因。猪胚胎在妊娠第 10~12 天之间经历从球状到管状和丝状的快速转变<sup>[25]</sup>, Isom 等<sup>[26]</sup>发现 11.3% 的猪克隆胚胎在妊娠第 14 天仍然是球状,表明部分克隆猪胚胎由于形态转变失败而停止发育。克隆胚胎的胚盘(embryonic disc, ED)和滋养层(trophoderm, TE)与正常受精胚胎进行转录组比较,发现克隆胚胎的 ED 和 TE 中都有大量 DEGs,其中 ED 的 DEGs 主要参与基因表达的表观遗传控制及 microRNA 介导的基因沉默和细胞凋亡,TE 的 DEGs 主要与异常的代谢/分化途径和亚细胞的组织缺陷有关<sup>[26]</sup>。因此,即使胚胎能够附植成功,但大部分

克隆胎儿和胎盘的发育仍然存在缺陷。Ruan 等<sup>[16]</sup>在对妊娠 30 天和 35 天猪克隆胚胎进行转录组研究中发现,与 AI 胚胎相比,异常克隆胚胎中表达下调的基因数量比正常克隆胚胎更多,且大多数与胚胎发育相关的基因在异常克隆胚胎中未能激活表达。此外,大多数克隆猪具有宫内发育迟缓(intrauterine growth retardation, IUGR)特征,妊娠 65 天的克隆猪体重以及足月初生重显著低于 AI 猪<sup>[5,19,27]</sup>。初生重是一个重要的新生儿发病预测指标,克隆猪的初生重低可能是其出生后高频率死亡的重要原因<sup>[5]</sup>。

## 2 克隆猪发育异常的原因

### 2.1 表观重编程错误

目前,供体核的表观重编程错误成为克隆胚胎发育异常的主流观点,主要包括 DNA 甲基化、组蛋白修饰和 X 染色体失活异常<sup>[28]</sup>。很多研究团队已经尝试通过修正这些表观遗传修饰错误来改变克隆胚胎的命运。DNA 甲基化重塑是早期胚胎发育的关键步骤,涉及 DNA 去甲基化和再甲基化,由 DNA 甲基转移酶(DNA methyltransferase, DNMT)的催化作用完成<sup>[29]</sup>。供体细胞是克隆胚胎发育的起点,其表观修饰状态会直接影响克隆胚胎的发育能力<sup>[30]</sup>。研究发现,骨髓间充质干细胞(bone marrow stroma cell, BMSC)的囊胚率显著高于胎儿成纤维细胞(fetal fibroblasts, FF),可能由于 BMSC 来源的胚胎具有更低的 *Nanog* 和 *Pou5f1* 基因启动子 DNA 甲基化水平、更高的 H3K9Ac 水平和更低的 H3K9me3 和 5-甲基胞嘧啶水平<sup>[31]</sup>。因此,不同类型的供体细胞的克隆胚胎发育效率具有明显差异,这很可能与供体细胞的分化程度有关<sup>[11,32]</sup>。DNA 甲基化重塑异常几乎是所有克隆动物共有的特征,包括去甲基化不完全和再甲基化异常<sup>[33,34]</sup>。附植前胚胎的 DNA 甲基化需要经历特定的变化,其中早期和后期囊胚具有相似的 DNA 甲基化水平,且后期囊胚内细胞团(inner cell mass, ICM)的 DNA 甲基化水平显著高于 TE<sup>[34]</sup>。然而,早期克隆囊胚的 DNA 甲基化水平显著高于后期囊胚,且后期囊胚的 ICM 与 TE 的 DNA 甲基化水平没有差异,表明 DNA 甲基化印记的异常变化可能造成胎儿和胎盘的发育缺陷<sup>[35]</sup>。最近, Gao 等<sup>[36]</sup>研究

发现附植前克隆胚胎经历异常 DNA 再甲基化,通过特定的 DNMT 抑制剂使 DNA 甲基化恢复到正常水平显著提高了小鼠(*Mus musculus*)的克隆效率,表明异常的 DNA 再甲基化也是抑制克隆胚胎发育的重要原因。DNA 甲基化异常可能与 *Dnmt* 基因表达失调有关,抑制 *Dnmt* 基因的表达降低克隆胚胎中异常的高甲基化水平而利于基因的转录激活。研究发现, DNMT 抑制剂 RG108 能促进 DNA 的主动和被动去甲基化以及 *Nanog* 转录而增强附植前克隆猪胚胎的发育能力<sup>[29,37]</sup>。对妊娠中期克隆胎儿流产的研究发现, 胎儿的 DNA 重复区域 *PRE-1* 和卫星序列都呈现高度甲基化, 且胎儿和胎盘中很多印记基因表达异常以及 *H19* 的 DMR3 处于低甲基化水平, 表明胎儿和胎盘发育异常都可能造成流产<sup>[38]</sup>。此外, DNA 甲基化变化可能与克隆仔猪的异常表型有关。出生后的异常克隆猪与正常受精猪在全基因组的基因表达模式和 DNA 甲基化水平存在明显差异<sup>[39]</sup>, 且异常克隆猪的全基因组相对正常克隆猪更多是呈现低 DNA 甲基化水平<sup>[40]</sup>。然而, 不能定论异常克隆仔猪的全基因组都是处于低 DNA 甲基化水平, 因为其 CpG 岛区域具有更高的 DNA 甲基化水平<sup>[40]</sup>。尽管表型正常的克隆猪与普通猪的基因表达模式高度相似, 但是克隆猪呈现更多不同的单拷贝序列 DNA 甲基化模式, DNA 甲基化水平的差异可能会影响克隆猪的组织或器官发育<sup>[41]</sup>。

哺乳动物基因组的组蛋白的 N 末端有很多修饰形式, 包括甲基化、乙酰化、磷酸化和泛素化等<sup>[42]</sup>。这些修饰可以影响组蛋白与染色质的相互作用而调控基因的转录<sup>[28]</sup>。常见的组蛋白修饰是甲基化和乙酰化。组蛋白乙酰化可以减弱组蛋白与 DNA 的相互作用而促进基因转录, 然而组蛋白甲基化以残基的修饰位点而决定基因的转录和抑制<sup>[43]</sup>。如同正常的猪受精胚胎, 猪克隆胚胎的 H3K27 乙酰化水平从原核期到 8-细胞期逐渐降低, 这个时期对应胚胎的基因组激活, 但是在随后的发育中 H3K27 乙酰化异常<sup>[44,45]</sup>。另外的研究显示, 猪克隆胚胎原核或 2-细胞阶段的 H3K18 乙酰化水平与随后的发育能力呈正相关<sup>[46]</sup>。目前, 已有很多研究利用组蛋白去乙酰化抑制剂(histone deacetylase inhibitor, HDACi)调控猪克隆胚胎的组蛋白乙酰化水平而增强胚胎的发育能

力, 如 Trichostatin、Scriptaid、oxamflatin、MGCD0103、丁酸钠和丙戊酸等<sup>[30]</sup>。然而, 这些方法并不能提高猪克隆胚胎的体内发育效率。在猪中, 1-细胞到 4-细胞阶段克隆胚胎的 H3K9me2、H3K9me3 和 H4K20me3 的表达水平异常高于体外受精胚胎, 表明 H3K9me2、H3K9me3 和 H4K20me3 都有可能是猪克隆胚胎发育的表观障碍<sup>[47]</sup>。利用组蛋白甲基转移酶抑制剂 BIX-01294 可以显著降低 H3K9me2 水平并提高了胚胎的体内和体外发育效率<sup>[48]</sup>。H3K27me3 也被发现是猪克隆胚胎发育的重要表观障碍, 降低 H3K27me3 的水平能提高胚胎的发育效率<sup>[49]</sup>。然而, 早期胚胎阶段改变组蛋白修饰并不能保证胚胎体内的长期发育。最近研究表明, 附植前胚胎中 H3K27me3 的印记丢失可能是胚胎附植后发育缺陷的主要原因<sup>[50]</sup>。

此外, X 染色体失活异常也是克隆胚胎中的重要表观重编程壁垒。X 染色体失活是一种雌性特异的剂量补偿机制, 由 X 染色体连锁的父源等位基因非编码 RNA *Xist* 调控完成。在发育异常的克隆动物中, *Xist* 基因异常活化可能会造成胚胎致死或者流产<sup>[51]</sup>, 通过敲除或敲低 *Xist* 能够将小鼠的克隆效率提高 8~12 倍<sup>[52,53]</sup>。最近研究发现, 妊娠 30 天和 35 天的异常克隆胎儿的 *Xist* 基因异常高表达, 通过敲除供体细胞的 *Xist* 基因能将猪的克隆效率提高 6.9 倍, 表明 *Xist* 表达失调与克隆胎儿发育异常有关<sup>[16]</sup>。

## 2.2 胎盘发育缺陷

胎盘作为连接母体与胎儿的重要桥梁, 对胎儿的生长和发育具有重要调控作用。胎盘发育缺陷往往与多种妊娠并发症相关, 如先兆流产、IUGR、妊娠糖尿病和高血压等, 是导致胎儿发育不良甚至死亡的重要原因。猪胎盘属于上皮绒毛膜胎盘, 胚胎需要通过绒毛膜形成褶皱和内陷加大与子宫内壁的接触面积才能从母体循环摄取充足的营养物质<sup>[54]</sup>。对克隆猪的相关研究发现, 相对于同期的人工授精胚胎, 早期猪克隆胚胎的胚外组织形态异常, 妊娠中期和足月胎盘的褶皱、滋养层及血管化发育不良<sup>[5,23,26,55,56]</sup>; 胎盘中调控细胞凋亡、氧化应激、血管形成、细胞增殖等过程的重要基因表达及信号通路异常, 这些都可能是克隆猪宫内发育不良的重要



原因<sup>[5,57]</sup>。另外,最近研究发现,克隆猪宫内发育迟缓或发育不良很可能与胎盘皱褶发育缺陷、胆汁酸转运和类固醇激素合成相关基因表达异常及脂肪酸转运蛋白 4 表达下调有关<sup>[27,56]</sup>。此外,脐带异常影响血流而抑制胎儿的生长。Ao 等<sup>[5]</sup>研究数据显示,32.6% (15/46)存活的新生克隆猪的脐带畸形,表现为脐带膨大和闭塞性血栓,这些仔猪在出生后 4 天内基本死亡,因为脐带畸形抑制血管的收缩和降低血流,增加胎儿发育异常的风险。克隆猪的脐带发育异常可能与血管形成相关基因 *Vegf*、*Vegfr1*、*Ang1* 和 *Ang2* 的表达下调,以及参与抗氧化应激和调控糖酵解的蛋白表达水平下调及细胞凋亡相关蛋白表达上调密切相关<sup>[8]</sup>。目前,胎盘发育缺陷仍然是 SCNT 发展中的一个主要障碍。因为即使通过敲除 *Xist* 和过表达 *Kdm4a* 将小鼠的克隆效率提高到 20%,但是存活胚胎中仍然有胎盘异常的个体<sup>[50]</sup>。

### 3 提高猪克隆效率的有效方法

#### 3.1 寻找猪克隆胚胎发育失败的关键因子

供体细胞是生产克隆后代的遗传基础,供体核能否充分开启转录组重编程将很大程度决定 SCNT 胚胎的发育命运<sup>[58]</sup>。同济大学高绍荣课题组结合胚胎活检与单细胞测序方法对早期克隆胚胎进行了详细的转录组分析,发现 H3K9me3 去甲基化酶基因 *Kdm4b* 和 *Kdm5b* 分别在 2-细胞和 4-细胞期发育停滞的克隆胚胎中未被激活,过表达 *Kdm4b* 和 *Kdm5b* 能够恢复这两个发育阶段的转录谱而显著提高小鼠囊胚率(>95%)及出生率<sup>[59]</sup>。早期克隆胚胎的发育相关基因的正常表达与供体细胞的再甲基化密切相关。近期,高绍荣团队通过绘制小鼠附植前克隆胚胎的全基因组 DNA 甲基化图谱,发现 SCNT 胚胎大范围的 DNA 区域存在异常的 DNA 再甲基化,这种异常成为 SCNT 胚胎中合子基因和部分逆转座子未能完全激活的关键障碍,通过抑制 DNA 甲基化酶和过表达组蛋白去甲基化酶都能显著提高克隆小鼠的出生率<sup>[36]</sup>。供体细胞的组蛋白修饰模式未能重编程到受精卵状态也会导致克隆胚胎发育失败。美国哈佛大学张毅课题组结合转录组测序和染色质免疫共沉淀数据分析基因组不同区域的组蛋白修饰与基因表

达的联系,发现供体细胞中 H3K9me3 是小鼠克隆胚胎发育的主要障碍,通过过表达 *Kdm4d* 或敲除 H3K9me3 甲基化酶基因 *Suv39h1/2* 以降低供体细胞的 H3K9me3 水平可将克隆效率提高 8 倍左右<sup>[60]</sup>。最近的研究显示,过表达 *Kdm6a* 同样也可以显著提高克隆小鼠的出生率<sup>[61]</sup>。每个物种调控 H3K9me3 的模式不同,所以在人(*Homo sapiens*)<sup>[62]</sup>和牛(*Bos taurus*)<sup>[63]</sup>中,分别过表达 *Kdm4a* 和 *Kdm4e* 才能显著提高克隆胚胎的发育能力。在猪中,通过过表达 *Kdm4a* 能显著下调克隆胚胎的 H3K9me3 水平而提高体外发育效率,但由于 *Xist* 的启动子区域富含 H3K9me3,所以过表达 *Kdm4a* 不能支持克隆胚胎的长期发育<sup>[13]</sup>。此外,过表达 *Kdm4b/4d* 没有改变克隆胚胎的 H3K9me3 水平和体外发育效率<sup>[64]</sup>。由此可见,需要深入解析早期猪克隆胚胎的表观重编程变化才能有助于寻找猪克隆胚胎发育失败的关键因子。

#### 3.2 提高卵母细胞的成熟质量

供体核在卵母细胞胞质被诱导激活,所以卵母细胞很大程度上决定了重构胚的发育能力。研究发现,体内成熟卵母细胞作为核移植受体构建的克隆胚胎的囊胚率和出生率均显著高于体外成熟卵母细胞<sup>[65]</sup>,且经产母猪来源的卵母细胞所获得的克隆胚胎体外发育效率明显高于后备母猪来源的卵母细胞<sup>[66]</sup>,表明卵母细胞的成熟质量是影响克隆胚胎的发育能力的重要因素。卵母细胞成熟指第一次减数分裂前期到第二次减数分裂中期的过程,主要体现在核成熟和胞质成熟,通常以第一极体排出作为核成熟的标志,胞质成熟表明卵母细胞具备受精能力和受精后的发育能力以及所需的物质和能量储备<sup>[67]</sup>。体内成熟的卵母细胞在卵泡环境中能够实现细胞核和胞质同步成熟,但是体外成熟的卵母细胞在体外培养体系中不能保证胞质与细胞核的同步成熟,胞质的不完全成熟是造成体外成熟卵母细胞发育能力低的重要原因<sup>[68]</sup>。转录组比较分析发现体内与体外成熟卵母细胞中参与转录、细胞周期、转运和细胞蛋白代谢等生物学过程的基因表达具有明显差异<sup>[69]</sup>。最近的一项研究已经表明,改善卵母细胞发育质量能显著提高克隆胚胎的体内发育效率,研究人员通过在卵母细胞体外成熟的培养基添加成纤维细胞生

长因子 2、白血病抑制因子和胰岛素样生长因子 1 显著提高了克隆猪的囊胚率和出生率, 窝均产仔数达到 9 头左右, 可能由于这些细胞因子使卵丘细胞具有不同的 MAPK 激活模式、增加卵丘细胞扩张以及加快卵母细胞和卵丘细胞之间胞浆突起物的分离, 进而提高了卵母细胞的发育质量<sup>[70]</sup>。卵泡液为卵母细胞的生长和成熟提供了适宜的微环境, 在体外培养基中添加卵泡液也成为提升卵母细胞成熟质量的重要途径。Zhao 等<sup>[71]</sup>发现卵母细胞体外培养基添加体内成熟来源的卵泡液的克隆胚胎发育效率显著高于未成熟卵泡液, 可能由于体内成熟卵泡液能提供更多促进卵母细胞成熟的蛋白质。卵母细胞在体外培养环境缺乏对自由基的清除能力, 造成氧化应激水平高而降低卵母细胞质量, 在体外培养基中添加自由基清除剂能够一定程度提高卵母细胞质量和重编程能力。研究发现, 褪黑素通过降低卵母细胞的氧化应激水平提高了 SCNT 及 IVF 胚胎的发育效率<sup>[72,73]</sup>。这些结果表明优化体外培养体系来提高卵母细胞成熟质量对于增强克隆胚胎的体内发育能力具有重要作用。

### 3.3 降低代孕母猪的流产率

在克隆猪生产中, 代孕母猪的怀孕率仅为 50% 左右, 妊娠失败的主要原因是克隆胎儿流产, 且主要发生在妊娠第 30~60 天<sup>[17]</sup>。克隆胎儿流产的主要原因是供体细胞的重编程错误。Zhang 等<sup>[38]</sup>发现克隆猪胎儿流产可能与胎儿和胎盘中印迹基因的表达异常及基因组重复区域的高甲基化有关。此外, 品种、胎次、移植胚胎数量和排卵时间都会影响代孕母猪的怀孕率。与供体细胞品种相同的代孕母猪可获得更高的分娩率, 胚胎与受体品种间的差异会增加流产的风险<sup>[74]</sup>。双侧输卵管移植的代孕母猪比单侧移植的妊娠率和产仔率有显著提高<sup>[75]</sup>。排卵前 24 h 进行胚胎移植的母猪怀孕率和克隆效率都显著高于排卵前 6 h<sup>[76]</sup>。最近, Yu 等<sup>[77]</sup>对诱导多能干细胞(Induced pluripotent stem cells, iPS)和成纤维细胞来源的克隆猪胎儿和胎盘进行全基因组 DNA 甲基化和转录组测序分析, 结果发现 iPS 克隆胎儿和胎盘的父本印迹基因 *Rtl1* 处于异常的高甲基化状态, 超表达 *Rtl1* 能降低 iPS 胎儿流产率而显著提高 iPS 细胞的克隆效率, 这些结果揭示 *Rtl1* 的表达沉默很

可能是克隆胚胎的流产主要原因。此外, 日粮中添加营养物质对于降低代孕母猪流产率具有一定作用, 本课题组前期尝试在妊娠第 12~70 天代孕母猪日粮中补充精氨酸, 结果表明这种方式可以显著提高代孕母猪的怀孕率(62.9% vs. 44.5%)<sup>[78]</sup>。因此, 提高克隆胚胎的发育质量、选择适合的克隆胚胎移植方式以及调控克隆代孕母猪的营养水平都是降低代孕母猪流产率的重要途径。

## 4 结语与展望

克隆猪的宫内发育异常体现在胎儿和胎盘两部分, 核心是供体核表观重编程, 这里面涉及一系列复杂的表观遗传修饰变化。尽管供体核的表观重编程错误被认为是克隆胚胎发育失败的主要原因, 但是其机制仍然不清楚。因此, 需要系统和精细的分析重编程过程中染色体和表观基因组的变化。

组蛋白修饰异常是克隆胚胎发育失败的重要原因, 因而解析蛋白质与 DNA 的相互作用是一个重要的研究方向, 其中染色质可接近性与基因表达调控密切相关。目前, 已有多种技术研究人和小鼠附植前胚胎染色质可接近性而揭示胚胎发育过程中开放染色质的调控规律, 包括低通量脱氧核糖核酸酶 I 超敏感位点测序(low-input deoxyribonuclease I hypersensitive site sequencing, liDNase-seq)技术<sup>[79,80]</sup>和转座酶探究可接近性染色质高通量测序(assay for transposase-accessible chromatin with high-throughput sequencing, ATAC-seq)技术<sup>[81,82]</sup>。此外, 基于高通量测序的染色质构象捕获(high-throughput/resolution chromosome conformation capture, Hi-C)技术可以在全基因组范围内研究染色质的空间构象并揭示基因组的动态变化<sup>[83]</sup>。靶向调控供体细胞和克隆胚胎的表观基因组是更加有效地提高克隆胚胎的发育质量的一种途径<sup>[84]</sup>。目前, 很多研究已经实现了基于 CRISPR/Cas9 的靶向表观修饰。例如, Liu 等<sup>[85]</sup>构建了 Tet1、Dnmt3a 与失活的 Cas9(dCas9)的融合蛋白可实现 DNA 甲基化的靶向编辑。CRISPR-dCas9-SunTag-p300core 系统能够靶向重塑多能基因 *Oct4* 的启动子和增强子, 并且同时调控多个基因的表达<sup>[86]</sup>。这些技术的应用有助于研究人员解析供体核的表观重编程过程, 并解析猪克隆胚胎宫内

发育异常的分子机制。今后猪 SCNT 技术更应注重克隆胚胎的体内发育, 提高出生率与健康率才能更好地发挥克隆猪的应用价值。

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