

# 鸽乳的生物学功能及其生成调控

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**摘要:** 鸽(*Columba livia*)是少数几种能分泌营养液哺育雏鸟的鸟类之一。孵化期的亲鸽嗉囊壁逐渐增厚, 当雏鸽被孵出, 亲鸽嗉囊会产生鸽乳(crop milk)以哺育雏鸽。鸽乳的营养成分及其生物学功能与哺乳动物的乳汁相似, 其产生过程受催乳素的调节。在催乳素作用下, 嗉囊上皮细胞快速增殖脱落形成鸽乳, 该过程可能与膜联蛋白 Icp35(*AnxIcp35*)等关键基因的转录以及 JAK/STAT 和 Wnt 等信号通路的激活有关。本文对鸽乳的主要成分、生物学功能和泌乳过程中嗉囊组织学变化进行了介绍, 对鸽乳生成过程中特异的基因变化和分子调控机制进行了总结, 以期后续的相关研究工作提供有益的参考。

**关键词:** 鸽乳; 嗉囊; 鸽; 催乳素

## The biological function of pigeon crop milk and the regulation of its production

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**Abstract:** The pigeon (*Columba livia*) is one of the few birds capable of secreting nutrients to nourish squabs. During the incubation period, the crop of the parent pigeon will be thickened. When squabs are hatched, the crop milk will be secreted from the crop and fed to squabs. The nutritional benefits are similar between the pigeon crop milk and mammalian milk, and both of them are regulated by prolactin. Prolactin stimulates the proliferation of crop epithelial cells, which eventually slough to form the crop milk. Evidence suggests that the complex process may be associated with the transcription of the *AnxIcp35* gene and the activation of JAK/STAT and Wnt signal pathways. In this review, we summarize the main components and the biological function of the crop milk, the histological changes of the crop and the regulatory mechanism of crop milk secretion.

**Keywords:** crop milk; crop; pigeon; prolactin

收稿日期: 2017-04-10; 修回日期: 2017-06-24

基金项目: 四川省科技厅应用基础计划项目(编号: 2016JY0167), 四川省教育厅重点项目(编号: 15ZA0008)和四川省青年科技创新研究团队项目(编号: 2015TD0012)资助[Supported by the Application Basic Research Plan Project of Sichuan Province (No.2016JY0167), the Key Project of Sichuan Education Department (No.15ZA0008), and the Program for Innovative Research Team of Sichuan Province (No.2015TD0012)]

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DOI: 10.16288/j.ycz.17-132

网络出版时间: 2017/11/10 15:53:03

URL: <http://kns.cnki.net/kcms/detail/11.1913.R.20171110.1553.002.html>

鸽(*Columba livia*)作为人类较早驯化的鸟类之一,除肉、蛋、观赏及比赛等用途外,还是一种重要的实验动物被广泛用于监测大气污染<sup>[1]</sup>、构建动脉粥样硬化病理模型<sup>[2]</sup>以及辅助医生对肿瘤组织病理切片等进行诊断<sup>[3]</sup>。一直以来,鸽作为少数几种嗉囊可分泌营养液的鸟类之一,受到人们广泛的关注。鸽作为晚成鸟,雏鸽刚孵化出来时不能自主觅食,需依靠亲鸽哺育营养液——“鸽乳”(crop milk)存活<sup>[4]</sup>。鸽乳是雏鸽早期唯一的营养来源,研究鸽乳的生物学功能和亲鸽嗉囊分泌鸽乳的调节机制对于进一步深入挖掘鸽乳的应用价值以及揭开启动嗉囊分泌鸽乳的谜团具有重要意义<sup>[5]</sup>。因此,本文通过已有的相关研究,对鸽乳的主要成分、生物学功能和嗉囊的组织学变化进行介绍,并对鸽乳生成和作用过程中特异的基因变化和分子调控机制进行综述。

## 1 鸽乳的主要成分及其生物学功能

1786年,John Hunter首次报导了关于鸽乳的研究,其描述鸽乳为“白色颗粒状凝乳”,这种凝乳状物质产生于亲鸽的嗉囊中用以哺育幼鸽<sup>[4]</sup>。鸽乳中含有丰富的营养物质,是幼鸽生长发育所必需的营养来源,具有促进幼鸽生长发育、增强机体免疫力和调节肠道微环境等作用。

### 1.1 主要成分

鸽乳的主要成分包括蛋白质、脂肪、维生素和矿物质,其中蛋白质和脂肪含量最为丰富,分别占到总成分的11%~23%和4.5%~12.7%,其能量达到5.6~6.8 kcal/g<sup>[6~14]</sup>。随着雏鸽日龄的增长,鸽乳中蛋白与脂肪含量总体呈下降趋势<sup>[14,15]</sup>。鸽乳蛋白中富含生物活性酶和免疫球蛋白,包括大量的淀粉酶、蛋白酶、脂肪酶和肽酶等消化酶类,以及IgA、IgG、IgY、生长因子、转铁蛋白等<sup>[4,14,16~20]</sup>。氨基酸成分研究显示鸽乳中富含谷氨酸、天冬氨酸和亮氨酸,而蛋氨酸、色氨酸、组氨酸和半胱氨酸则相对较少<sup>[21]</sup>。鸽乳中脂质的主要成分包括甘油三酯、磷脂、胆固醇、游离脂肪酸、胆固醇酯和双甘酯。脂肪酸分析结果显示,鸽乳中含大量的脂肪酸,包括油酸、亚油酸、棕榈酸等<sup>[5,15]</sup>。除以上成分外,鸽乳中还含有维生素C、维生素A以及维生素B2,但缺

乏维生素B1<sup>[11]</sup>。

### 1.2 促生长作用

鸽乳具有促进生长的作用。雏鸽出生后饲喂鸽乳3周,其体重可增长近22倍<sup>[14]</sup>,鸡和小鼠的饲喂实验也表明每天饲喂少量鸽乳可提高它们的生长水平<sup>[11,22,23]</sup>。该过程中,鸽乳丰富的营养物质满足了幼鸽前期生长发育所需的营养需求,并且鸽乳中大量具有生物学活性的消化酶亦可能会提高雏鸽消化能力。1992年,Shetty等<sup>[19]</sup>鉴定出鸽乳中存在鸽乳生长因子(pigeon milk growth factor, PMGF),并通过分析发现其与表皮生长因子(epidermal growth factor, EGF)有相似的分子量峰值和洗脱模式,暗示该因子具有促进雏鸽快速生长的能力。给新生小鼠注射鸽乳水提物可促使小鼠门齿萌发和提前张眼<sup>[24]</sup>,同时鸽乳粗提物还有刺激仓鼠卵巢细胞增殖的能力<sup>[19,23]</sup>。此外,亚油酸(linoleic)和亚麻酸(linolenic acids)作为必需的多不饱和脂肪酸,在出雏1~5天鸽乳中的含量远远高于人初乳,它们涉及细胞膜脂流动性并参与磷脂的合成<sup>[25~27]</sup>。亚油酸和亚麻酸的缺乏会导致发育迟缓并影响婴儿的记忆力和思维能力。因此,鸽乳中高比例的亚油酸和亚麻酸在促进雏鸽快速发育过程中可能起到了一定作用。

### 1.3 调控肠道微环境

在哺育雏鸽的过程中,鸽主要通过逆呕的方式将嗉囊内的鸽乳和部分食物饲喂给幼鸽。由于鸽乳中大量微生物的存在<sup>[28]</sup>,哺育过程中亲鸽体内的微生物可通过鸽乳传递给雏鸽,从而影响雏鸽肠道微生物群的建立。在小鸡饲喂鸽乳的实验中,研究者发现饲喂鸽乳后小鸡盲肠菌群发生显著的变化,原本存在于鸽乳中的4个菌种(*Veillonella* 菌属)也在饲喂鸽乳的小鸡盲肠中被发现<sup>[29]</sup>。益生元作为一种不被消化的食物成分,可选择性促进一种或多种细菌的增殖或增加活性,有益于宿主的健康<sup>[30]</sup>。对人乳和猪乳的研究显示,乳汁中含有特定的益生元,其可导致母乳喂养和哺喂配方奶粉的婴儿和小猪的肠道微生物发生显著的变化<sup>[31,32]</sup>。而Shetty等<sup>[12]</sup>在第二周的鸽乳中亦发现低聚糖——一类潜在的益生元存在,并且饲喂实验提示鸽乳中富含的益生元可促进鸡盲肠乳酸杆菌种群的多样化<sup>[29]</sup>。上述研究表明

幼鸽通过摄入鸽乳获得的微生物及益生元有助于建立一个良好的肠道微环境。

#### 1.4 免疫作用

与哺乳动物相似, 鸽乳中富含 IgA、IgG、IgY 等免疫球蛋白<sup>[16~18]</sup>。这些免疫球蛋白的摄入对增加雏鸽免疫力具有重要作用。Goudswaard 等<sup>[16]</sup>通过标记鸽乳中 IgA, 发现 IgA 能有效传递至 1~3 日龄的雏鸽, 并通过胞饮的方式被吸收。机体的免疫力同时还受到肠道微生物的调节, 在无菌小鼠中, 其 CD4<sup>+</sup> T 细胞显著低于正常小鼠, 而这种影响可通过肠道菌群的接种得到有效的缓解<sup>[33]</sup>。肠道淋巴组织的免疫能力亦可通过改善肠道微生物来调节, 小鸡在饲喂鸽乳后, 其肠道淋巴组织中免疫相关通路的基因表达显著增加<sup>[29]</sup>。因此鸽乳亦可能通过改善雏鸽肠道微生物来进一步提高雏鸽的免疫能力<sup>[29,34]</sup>。近年来, 关于哺乳动物乳汁外泌体的研究显示大量免疫相关的 miRNA 高度富集在乳汁外泌体当中, 暗示这些乳汁中的外泌体可通过胃肠道进入幼体体内发挥免疫作用<sup>[35,36]</sup>。然而, 目前鸽乳外泌体的相关研究还未见报道。关于鸽乳中是否存在外泌体并通过 miRNA 在幼鸽体内发挥作用, 这一问题值得人们进一步探索。

## 2 嗉囊的组织学变化及鸽乳生成

尽管鸽乳在组成成分和功能上与哺乳动物乳汁存在一定的相似性, 并且都主要受到催乳素的调节, 但二者的分泌部位及形成过程则完全不同<sup>[37,38]</sup>。在结构上鸽嗉囊不具备分泌腺体, 这种特有的分泌鸽乳的能力主要由催乳素介导的嗉囊上皮细胞快速增殖脱落所致<sup>[39~42]</sup>。并且更有趣的是, 不仅雌鸽嗉囊可以分泌鸽乳, 雄鸽也可分泌<sup>[43]</sup>。

#### 2.1 嗉囊的结构及泌乳周期的变化

嗉囊普遍存在于大部分鸟类中, 其位于食管远端和腺胃近端之间, 是食管进入胸腔之前的一个囊状膨大憩室<sup>[44]</sup>。通常嗉囊壁较薄, 且具有很大的延展性, 主要起到储存、润湿、软化、发酵食物并控制食物传递到前胃和胃的速度以达到延长食物供给和充分研磨消化的作用<sup>[38,45,46]</sup>。除以上功能外, 鸽及鸠鸽科主要成员的嗉囊能分泌鸽乳以哺育雏鸟。

在不同繁殖阶段, 嗉囊组织结构有着不同的变化。在休产期, 鸽嗉囊壁薄而透明, 而随着孵化的进行, 嗉囊呈现上皮细胞快速增殖及嗉囊壁逐渐增厚的变化<sup>[47,48]</sup>。在该过程中, 结缔组织和血管增生, 为细胞增殖及营养物质的积累提供丰富的血液供应<sup>[46]</sup>。同时嗉囊基底层细胞和棘层的深层细胞的分裂不断增强, 并往上推移, 导致嗉囊上皮增厚; 棘层的浅层细胞和表面的扁平细胞内脂类物质的逐渐合成、积累和贮存, 加之血液供应不足, 导致细胞变性, 最终形成累积脱落的营养细胞层, 落入嗉囊腔形成鸽乳<sup>[49]</sup>。

#### 2.2 鸽乳的脂质产生与调节

鸽乳干物质成分主要由蛋白质和脂肪构成, 而在整个“泌乳”周期内, 鸽乳中甘油三酯占总的脂质比例从 81.2%(1 天)下降到 62.7%(19 天), 随着“泌乳”过程的进行, 细胞内蓄积的脂质含量逐渐降低而细胞膜相关的脂质含量却保持不变<sup>[15]</sup>。早在 1975 年, Garrison 和 Scow<sup>[50]</sup>通过对成年鸽嗉囊注射催乳素, 嗉囊呈现增重及脂蛋白脂肪酶(lipoprotein lipase, LPL)活性显著增加。而 LPL 在甘油三酯的水解和吸收过程中起到至关重要的作用, 提示鸽乳中含有的脂质可能来源于血液的供给<sup>[51]</sup>。1984 年, Horseman 和 Will<sup>[52]</sup>在研究鸽嗉囊过程中未检测到脂肪合成相关的证据, 进一步证实嗉囊中存在的大量脂质可能来自循环系统的运输。而且来自肝脏和脂肪的氧化型甘油三酯可以通过极低密度脂蛋白(very low density lipoprotein, VLDL)运输并以内吞的方式进入细胞内, 而内吞作用信号通路相关的基因亦在“泌乳”的嗉囊组织中高度富集<sup>[46]</sup>。然而, 近年来在哺乳动物乳腺中的研究表明 LPL 在重新合成甘油三酯过程中显著上调<sup>[53~55]</sup>, 暗示鸽乳中脂质的产生很可能是在重新合成和血液运输共同作用下形成。Gillespie 等<sup>[4]</sup>通过高通量测序的方法鉴定 LPL 基因在泌乳嗉囊组织中高表达, 同时通过比较小鼠乳腺组织中脂质合成相关基因在泌乳鸽嗉囊中的表达情况, 结果显示 34 个小鼠乳腺脂质合成有关的基因在鸽泌乳嗉囊中也呈现差异性表达。并且涉及脂肪酸合成相关的 ELOVL 基因家族在小鼠乳腺和鸽嗉囊中表达存在差异。ELOVL6 具有促进长链脂肪酸 C16:1、C18:1、C18:2 合成的作用, ELOVL6 基因在泌乳嗉囊中高表达表明鸽乳中这类主要的长链脂肪

酸可在腺囊中重新合成<sup>[4,56]</sup>。相较于哺乳动物而言, 鸽乳中缺乏超长链的脂肪酸, 而 *ELOVL1* 则具有促进超长链脂肪酸合成的作用, 因此该过程可能与 *ELOVL1* 基因在哺乳动物泌乳期高表达有关<sup>[4,56]</sup>。

### 2.3 鸽乳的蛋白合成与调节

对哺乳动物的研究显示 mTOR 信号通路在乳汁蛋白的生成过程中起到重要作用<sup>[57]</sup>。mTOR(mammalian target of rapamycin)是雷帕霉素在哺乳动物细胞内作用的蛋白激酶<sup>[58,59]</sup>, 其可以通过对下游效应蛋白(4EBP1, p-70S6K)磷酸化水平的改变来调节乳汁蛋白的合成<sup>[60]</sup>。Hu 等<sup>[61]</sup>比较泌乳前后鸽腺囊的 IRS1/AKT/TOR 信号通路相关蛋白的磷酸化水平, 其结果显示鸽乳中的蛋白合成与哺乳动物相似, 亦受到 IRS1/AKT/TOR 信号通路的调节。此外, 在哺乳动物中, 催乳素可通过 JAK/STAT 信号通路与乳蛋白相关基因(乳清酸、酪蛋白等)启动子上的 GAS 序列(TTCNNNGAA)结合, 从而促进乳蛋白基因的表达<sup>[62]</sup>, 因此催乳素介导的 JAK/STAT 信号通路也可能参与了鸽乳中蛋白的合成。热应激蛋白家族作为细胞应激过程中的标记蛋白, 在多种类型的细胞应激反应(包括炎症和低氧应激过程)中均呈现高表达<sup>[63]</sup>, 并且在促进蛋白质折叠和运输过程中亦起到重要作用<sup>[64]</sup>。Gillespie 等<sup>[4]</sup>研究发现鸽乳中富含高比例蛋白也可能与热应激蛋白在泌乳腺囊中高表达有关。

## 3 催乳素调控腺囊分泌鸽乳的分子机制

大量的组织形态学研究已表明鸽乳是由脱落的腺囊上皮细胞产生, 而这一过程则主要与催乳素作用于腺囊生发层引起的细胞快速增殖有关。催乳素作为一种垂体前叶肽类激素, 主要受下丘脑催乳素释放因子调节以维持催乳素在动物体内的基础分泌<sup>[65,66]</sup>。已有的研究表明, 催乳素水平会随着机体生理和病理的状态而发生变化, 同时亦受到光照等外界刺激的影响, 并且单胺类递质包括多巴胺和 5-羟色胺亦具有促进催乳素分泌的作用<sup>[66~68]</sup>。但与哺乳动物不同的是, 雄鸽亦具有分泌鸽乳的能力。泌乳周期内, 雄鸽分泌鸽乳的机制还有待人们进一步研究。

催乳素在调节脊椎动物繁殖、泌乳、生长发育和行为等过程发挥重要作用<sup>[69,70]</sup>, 包括其最重要的

促进哺乳动物乳汁分泌的功能<sup>[71]</sup>。20 世纪 30 年代, 人们首次在鸽体内发现并提纯了催乳素<sup>[72]</sup>, 随后的研究显示给非泌乳期的家鸽注射催乳素会导致腺囊组织呈现与“泌乳期”相似的形态学特征<sup>[73]</sup>, 并且注射催乳素后腺囊组织的 mRNA 和蛋白水平亦会发生改变<sup>[74,75]</sup>, 甚至在幼鸽断“乳”前注射催乳素亦会促进其腺囊的发育和分化<sup>[76]</sup>。在整个繁殖周期内, 鸽血浆催乳素水平和腺囊重量的变化也存在相关性<sup>[38]</sup>。尽管在生殖周期内雄鸽的催乳素水平普遍低于雌鸽, 但二者催乳素水平的变化规律一致<sup>[77]</sup>, 而且繁殖周期内不同时间点雌雄鸽腺囊基因的表达亦无明显的差异。

催乳素受体作为细胞因子受体家族的成员, 位于细胞膜表面。与哺乳动物不同的是, 鸟类的催乳素受体包含两个完整的细胞外配体结合域。对同属于鸚鵡科的斑鸚的研究结果显示, 催乳素在细胞膜上与其受体结合后, 可能通过激活 JAK 信号通路进而促进 STAT 蛋白的磷酸化影响膜联蛋白 Icp35 (*AnxIcp35*)和其他潜在基因的转录(图 1)<sup>[38,78]</sup>。*AnxIcp35* 作为最丰富的催乳素介导腺囊基因产物, 其 mRNA 水平可在催乳素作用下 2 小时呈现快速的增加<sup>[79]</sup>。与此同时, 鸽还具有另外一种膜联蛋白 I 基因(*AnxIcp37*)<sup>[80,81]</sup>, 而且二者具有 93% 的序列相似性<sup>[82]</sup>。*AnxIcp37* 普遍存在于各个组织中, 但是 *AnxIcp35* 则在催乳素调节的腺囊中特异表达<sup>[80,81]</sup>。*AnxIcp35* 由于缺少一个酪氨酸激酶磷酸化位点, 其可以被催乳素激活并且不具备翻译后修饰, 而 *AnxIcp37* 则持续性表达并通过翻译后修饰激活<sup>[82]</sup>。间接证据显示 *AnxIcp35* 涉及多泡体(multivesicular bodies, MVB)和内含囊泡(endocytotic vesicles, ECVs)的合成和运输<sup>[38]</sup>, 并且由于膜联蛋白涉及内含功能, 暗示 *AnxIcp35* 蛋白在催乳素刺激下可能与细胞的内吞作用有关(图 1)<sup>[76,83,84]</sup>。

尽管催乳素调控腺囊分泌鸽乳的详细分子机制还未完全阐明, 但已有的证据显示催乳素可刺激腺囊的中间丝蛋白 CMP58 和 CMP50.5(crop milk polypeptides)、鸟氨酸脱羧酶(ornithine decarboxylase, ODC)、脂蛋白脂肪酶等发生特异性变化<sup>[52,79,85~86]</sup>。在催乳素作用下, 腺囊组织 CMP58 和 CMP50.5 的表达水平会显著提高, 二者可在脂滴周围形成类似

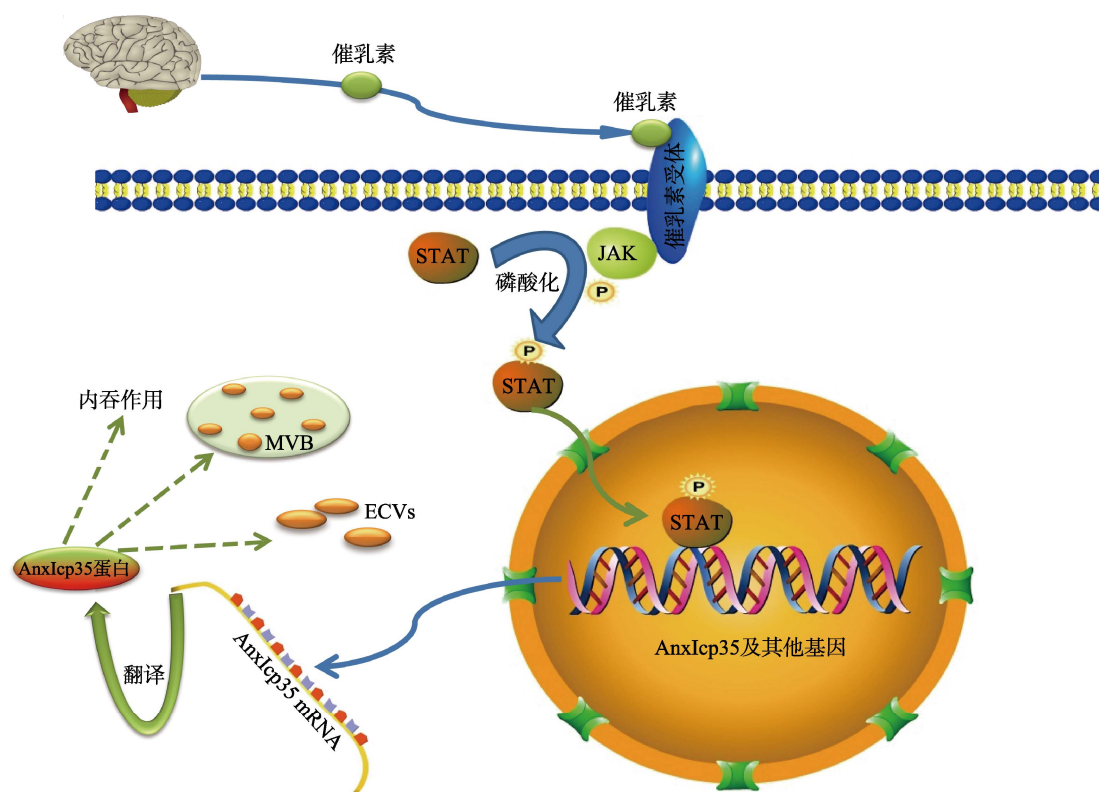


图 1 催乳素调节腺囊上皮细胞基因表达的潜在机制

Fig. 1 The potential mechanism for prolactin to regulate the gene expression in cropsac epithelial cell

参考文献[38,76,78,83,84]绘制。

于笼子的结构,从而使上皮细胞脂滴蓄积<sup>[52]</sup>。ODC 作为多胺合成中重要的限速酶,其活性与细胞增殖密切相关,在生长旺盛组织中的 ODC 活性显著高于生长缓慢或处于静止期的细胞和组织<sup>[87,88]</sup>。Nishiguchi 等<sup>[89]</sup>对人的颗粒细胞研究显示,催乳素可使其 ODC 活性增加,促进 DNA 合成和细胞增殖。因此泌乳期腺囊上皮细胞的快速增殖可能与催乳素刺激腺囊引起的鸟氨酸脱羧酶的活性增加有关。同时,在腺囊增殖和泌乳过程中,其他相关激素包括胰岛素,松弛素,多巴胺,血清素等亦可促进腺囊发育和鸽乳的形成<sup>[61,90,91]</sup>,与催乳素起到协同作用。

#### 4 泌乳周期内腺囊相关基因的变化

在整个泌乳周期内,鸽腺囊的形态学特征和组织结构会发生显著的变化,包括形成复杂的、高度折叠的上皮结构,营养细胞层的形成以及脱落形成鸽乳<sup>[41,42]</sup>。Gillespie 等<sup>[46]</sup>通过基因芯片分析鉴定出 542 个在泌乳腺囊组织中高表达基因和 639 个低

表达基因,并且通过 KEGG Pathway 和 GO 分析显示这些泌乳期的腺囊组织中上调的基因主要参与黑色素细胞增殖、细胞外基质-受体相互作用、黏附连接、Wnt 信号通路、抗氧化反应和微管运输等过程。鸽的腺囊生发层上皮细胞的增殖可能与黑色素生成通路的重要基因 *MITF*(microphthalmia-associated transcription factor)有关,*MITF* 基因在泌乳腺囊中上调<sup>[46]</sup>,该基因在哺乳动物中受到 MAPK(mitogen-activated protein kinase)和 Wnt 信号通路的调节<sup>[92,93]</sup>。对腺囊泌乳期研究发现快速增殖的生发层细胞会由于血液供给的缺乏导致缺氧<sup>[46]</sup>,而过氧化物酶 1 作为抗氧化蛋白<sup>[94]</sup>,其在腺囊组织中高表达可能起到了调节氧化还原平衡的作用。与此同时,许多免疫相关的基因亦在泌乳腺囊组织中上调<sup>[46]</sup>,包括在血小板中表达的趋化因子 *CXCL4*<sup>[95]</sup>,该基因暗示血小板在腺囊组织中存在渗透的情况。这些抗氧化和免疫相关基因在泌乳过程中上调,很有可能一方面起到保护亲鸽腺囊组织的作用,另一方面亦起到直接

促进后代免疫系统的发育<sup>[46]</sup>。

泌乳过程中鸽腺囊表皮增生和角质化形成鸽乳与泌乳期腺囊中角质化基因的差异表达有关<sup>[6]</sup>。培养鸡的角质化细胞显示  $\alpha$  角蛋白,  $\beta$  角蛋白以及角质化包膜前体基因 *Envoplakin* 和 *periplakin* 表达, 并且有中性脂肪的累积<sup>[96]</sup>。然而哺乳动物与鸟类不同, 它们可表达  $\alpha$  角蛋白却无法表达  $\beta$  角蛋白, 因此哺乳动物的角质细胞无法积累中性脂肪<sup>[96,97]</sup>。Gillespie 等<sup>[4]</sup>推测鸽类在进化过程中能产生鸽乳与鸟类角质细胞具有积累中性脂肪的能力有关。他们鉴定出鸽腺囊组织中角质化基因包括 *Cornulin*、S100-A9 和 A16 类似物、转谷氨酰胺酶 6 类似物以及鸽泌乳特异的膜联蛋白 AnxIcp 35, 并且发现几个与爪和鳞片相关的  $\beta$  角蛋白可能在腺囊泌乳过程中起到至关重要的作用<sup>[4]</sup>。

## 5 结语与展望

一直以来, 人类都十分关注亲鸽腺囊分泌鸽乳哺育雏鸽的独特生物学现象, 该现象背后隐藏的机制已初步得到阐明。但是, 基于研究手段、方法和资源的限制, 关于如何启动亲鸽泌乳, 尤其是雄鸽泌乳的机制以及这一特殊机制如何进化而来等诸多问题都还有待进一步探索。2013 年, Shapiro 等<sup>[98]</sup>完成了鸽类基因组的 *de novo* 测序, 并提供了鸽的参考基因组和编码蛋白质基因的注释文件; 2016 年, Damas 等<sup>[99]</sup>采用比较基因组结合 PCR 验证等方法进一步将鸽基因组 Scaffold N50 从 3.15 Mb 提高到 22.17 Mb, 并将基因组装配到染色体水平。以上两项重大研究进展为人们进一步研究鸽乳的分泌机制提供了宝贵的资源, 相信不久的将来, 关于亲鸽腺囊分泌“鸽乳”这一神奇生物学现象的机制将得到人们的进一步解析。

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