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性早熟动物造模研究进展

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【摘要】 性早熟是儿童常见内分泌疾病,动物实验在研究性早熟的发病机制和药物治疗中必不可少,因此建立合适的动物模型是研究性早熟的必要前提。啮齿类(如大鼠)与非人灵长类动物的青春期发育过程与人类近似,性腺发育指征明确,是性早熟模型常用动物。根据研究内容不同出现了达那唑、N-甲基-DL-天冬氨酸、雌二醇、高脂饲料、不良生活条件、光照及褪黑素、锰暴露、环境内分泌干扰物、颅脑辐射、脑内药物注射等多种性早熟造模方法。目前多采用阴道开口时间、阴道涂片及动情周期观察、血清性激素水平测定、下丘脑基因检测、性器官检测等多种指标对性早熟动物实验进行评估。

【关键词】 性早熟;中枢性性早熟;动物模型;造模方法;检测指标
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Advances in animal modeling of precocious puberty

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【Abstract】 Precocious puberty is a common endocrine disease in children. Animal experiments are essential in the study of the pathogenesis and drug treatment of precocious puberty. Therefore, establishing an appropriate animal model is a prerequisite for studying the condition. The pubertal development processes in rats and non-human primates are similar to those in humans, and the indications of gonadal development are clear. Rats and non-human primates are common animal models of precocious puberty. According to different research needs, there are many modeling method of precocious puberty, such as danazol, N-methyl-DL-aspartic acid, estradiol, high-fat feed, low-safety environment, light and melatonin, manganese exposure, environmental endocrine disruptors, brain radiation, and intracerebral drug injection. At present, vaginal opening time, vaginal smear and estrous cycle observations, serum sex hormone level determination, hypothalamic gene-level index detection, sexual organ detection, and others, are the most common indicators used to evaluate precocious puberty during animal experiments.

【Keywords】 precocious puberty; central precocious puberty; animal model; modeling method; detection index
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性早熟是一种以性发育提前为主要表现的内分泌疾病,女孩在 8 岁前、男孩在 9 岁前出现第二性征发育即为性早熟[1]。近年来全世界范围内性早熟的发病率逐年上升,且具有明显的种族及地域差异[2]。新冠疫情至今,线上教学及居家办公的推广,儿童户外运动减少,电子产品使用时间增加[3-4],儿童呼吸系统疾病发病率显著下降,性早熟及快速进展型青春期就诊率显著增加,性早熟已经成为继肥胖之后影响儿童健康的第二大问题,引起了研究者的重视[5]。目前研究发现性早熟的发病与遗传、情绪、饮食、肥胖、环境内分泌干扰物暴露等因素存在相关性[6-10]。性早熟的易感因素、发病机制及药物治疗的作用通路均需要通过动物实验验证,因此如何制作符合研究需要的性早熟动物模型尤为重要,故本文回顾相关文献,梳理不同性早熟造模方法、实验动物选择及评价指标以供研究者参考。

1 实验动物

中枢性性早熟是由下丘脑-垂体-性腺轴(hypothalamus-pituitary-gonadal axis, HPGA)功能提前启动引起。不同动物的中枢神经系统对垂体促性腺激素分泌的调节有很大差异。非人灵长类和啮齿类动物与人类 HPGA 调控机制相似,性发育均由下丘脑以间歇性脉冲形式促进 GnRH 分泌增加而激活,且性发育指征明显[11-13]。因此性早熟实验多选择啮齿类动物(大鼠为主)、非人灵长类(如食蟹猴)动物等。

1.1 大鼠

性早熟大鼠实验多选择 SD 大鼠、Wistar 大鼠。SD 大鼠、Wistar 大鼠繁殖力强、产仔多,生长发育快、性格温顺,SD 大鼠对性激素反应敏感,Wistar 大鼠性周期稳定,易受到外界的气温、气压、湿度、躁声等方面的影响,故二者常用于性早熟动物实验。一般开放性饲养条件下,大鼠寿命为 2.5 ~ 3 年,雄性大鼠性成熟约 2 月龄,雌性性成熟约 2.5 月龄,70 ~ 75 d 左右阴道开口,性周期 4 ~ 5 d[14]。

1.2 非人灵长类

非人灵长类动物在亲缘关系上和人最接近,与人类的遗传物质有 75% ~ 98.5%的同源性,具有很多与人类相似的生物学特征[15],其中,食蟹猴、猕猴及狨猴被认为是研究毒理学较好的非灵长类动物,而食蟹猴是其中最佳且应用最多的种属,食蟹猴及部分恒河猴的生殖内分泌与人类的生殖内分泌非常相似[16]。正常饲养条件下,雄性实验猴约 4.5 岁性成熟,雌性 3.5 岁性成熟,性周期 21 ~ 28 d,月经期 2 ~ 3 d,月经开始后 12 ~ 13 d 排卵[14]。最初人类月经周期的内分泌控制原理是用猕猴动物模型描述的,非人灵长类配子形成机制、受精、胚胎在子宫内着床和早期妊娠维持等生殖生物学方面与人有着众多相似之处[17]。相较于其他非人灵长类动物,食蟹猴体型较小、性格温顺、便于操作,因此部分性早熟实验将食蟹猴作为常用非啮齿类实验动物。

2 造模方法

目前研究者根据临床横断面研究、队列研究及病例对照研究发现了众多与性早熟相关的危险因素,制作出了多种性早熟动物模型,达那唑、N-甲基-DL-天冬氨酸(N-methyl-DL-aspartic acid, NMA)诱导法是临床较为常用的性早熟模型,本文将以啮齿类动物(大鼠为主)为例,探讨性早熟造模方法,具体造模方法对比见表 1。

2.1 达那唑诱导法

达那唑诱导法是选取 5 日龄雌性大鼠皮下注射 300 μg 达那唑(溶于 25 μL 体积比 1∶1的乙二醇/乙醇溶液)建立的真性性早熟模型。这种方法最早是由 Morishita 等[18]提出,当皮下注射 100 ~ 500 μg 达那唑时大鼠阴道开口时间、首次发情时间及正常发情周期均有显著提前,其中 300 μg 时效果最显著。达那唑是 17α-乙炔睾丸酮的衍生物,诱导的性早熟大鼠阴道脱落细胞学改变与成年大鼠一致[19],该方法可使下丘脑 KISS-1 mRNA 和吻素(kisspeptin, KP)的表达显著上调,下丘脑促性腺激素释放激素(gonadotropin-releasing hormone, GnRH) mRNA 表达增加,GnRH 的释放提前说明达那唑可加速 HPGA 的成熟速度,可能与 kisspeptin/G 蛋白偶联受体 54(G-coupled protein receptor 54, GPR54)信号通路有关[20]。此造模方法简单、造模周期短、持续时间长,性周期和正常青春期动物性周期完全一致。

2.2 NMA 诱导法

NMA 诱导法选取 26 日龄雌性大鼠,每日 14:00 和 16:00 皮下注射 NMA 40 mg/kg。NMA 是一种兴奋性氨基酸受体激动剂,通过诱导下丘脑促进 GnRH 分泌,从而促进促黄体生成素(luteinizing hormone, LH)分泌,使 HPGA 功能提前激活。国内

外研究证实,NMA 诱导的性早熟模型阴道开口及首次发情时间较正常组提前,规则性周期出现后的阴道涂片与正常青春期组大鼠阴道涂片表现相同,子宫指数、卵巢指数、卵巢黄体出现率和子宫壁厚度较正常组均有显著性差异,且下丘脑 GnRH mRNA 表达水平和血清 LH 水平也均高于正常组[21-22]。NMA 刺激分泌 LH 作用与年龄相关,从婴儿到青少年期,NMA 对 GnRH 神经元的控制作用逐渐增强[23-24]。Gore 等[25]发现,大鼠出生后 25 日龄左右开始的皮下 NMA 更易导致 GnRH mRNA 水平的加速升高和性早熟。该造模方法简便、有效,性发育特征与正常青春启动组的变化相同。

2.3　雌二醇(estradiol, E_2)诱导法

E_2 诱导法选取 15 日龄雌性大鼠,采用灌胃给予雌二醇 50 μg/kg,每天 1 次,连续 5 d 制备性早熟模型。杨嵘等[26]发现该造模方法较溶剂对照组相比阴门开启平均日龄、首次发情间期出现的平均日龄和性周期稳定后首次发情期出现的平均日龄均有明显提前,阴道指数相对升高 151%,GnRH、KISS-1、GPR54、GnRH mRNA 表达量及血清 LH、卵泡刺激素(follicle-stimulating hormone, FSH)和 E_2 水平均显著增加。雌二醇膜启动的信号传导促进下丘脑星形胶质细胞中的神经孕酮(neuroprogesterone, neuroP)合成,其作用于 E_2 诱导的孕酮受体以刺激 kisspeptin 释放,从而激活 GnRH 释放。实验证实下丘脑 neuroP 在性腺完整的雌性和用 E_2 治疗的卵巢切除大鼠中从青春期前增加到成年早期,E_2 促进黄体酮合成的青春期发育是促进生殖成熟的神经开关之一[27]。此方法操作简单、造模周期短,性发育特征与正常青春启动组的变化相同。

2.4　高脂饲料造模法

高脂饲料造模法较多:一种是对断奶后的雌性大鼠,持续给予高脂饲料喂养来制备性早熟模型[28];另一种是培育二代肥胖大鼠,给予哺乳期一代鼠及断奶后二代仔鼠高脂饲料制备性早熟模型,亦可选取肥胖型性早熟一代鼠全程给予高脂饲料繁育二代鼠,在二代鼠断奶后给予高脂饲料喂养制备性早熟模型[29]。大量临床研究证实肥胖是导致性早熟的高危因素[30-32]。研究发现高脂饲料喂养大鼠较正常饮食的大鼠提前进入青春期,高脂喂养的雌鼠所产仔鼠进入青春期的时间较正常饮食所产的仔鼠明显提前,断乳后继续高脂喂养会进一步加速这种趋势[33-35]。肥胖导致性早熟的发病机制可能与组织沉默调节蛋白 1(Sirtuin 1, SIRT1)介导的 KISS1 抑制[36]、kisspeptin/下丘脑室旁核(paraventricular nucleus of hypothalamus, PVN)神经酰胺/卵巢交感神经通路[37]、肠道微生物群诱导一氧化氮改变有关[38]。虽然此类模型应用研究较多,但诱导性早熟使用的高脂饲料的成分各不相同,目前没有统一标准。

2.5　不良生活条件造模法

Strzelewicz 等[39]将大鼠生活条件分为高资源条件、标准资源条件及低资源条件,高资源条件提供 91.5 cm × 64 cm × 159 cm 多层笼子,笼内温度保持在 20℃、12 h 的光/暗循环,随意获取食物和水、垫层、一根管子、一根咀嚼骨、筑巢材料、充足玩具,玩具位置和类型每周更换 2 次,以刺激新奇感;标准资源条件为 51 cm × 41 cm × 22 cm 的单层笼子,笼内温度和光照条件与高资源条件相同,并放置玉米垫、一根塑料管、一根嚼骨、筑巢材料和随意接触食物和水;低资源条件提供少量垫料及用纸代替筑巢材料。选取 36 只雌性和 12 只雄性大鼠,分别在标准资源条件下给与标准饮食饲养 2 周,共获得 36 只雌性和 10 只雄性实验大鼠。将 14 只雌性和 4 只雄性大鼠给予西方饮食饲养,雌鼠和幼鼠在 P2-10 期间移入低资源条件饲养;其余 22 只雌性和 6 只雄性大鼠在标准资源条件下给与标准饮食饲养,在雌性大鼠繁殖前 1 周,将 10 只雌性大鼠从标准资源条件移入高资源条件饲养,剩余 12 只雌性大鼠作为标准化组[39]。研究显示在低资源条件下给与西方饮食饲养成长的幼鼠青春期发育较其它两组明显提前,出现代谢功能障碍,如肥胖、性早熟和后代下丘脑 kisspeptin 系统破坏[39]。除上述方法制备性早熟模型外,还可在标准资源条件饲养的基础上,在幼鼠 PND 2 ~ 14 期间每天将所有幼鼠从笼中取出,放置在孵化器中 3 h,使其与雌鼠分离引起应激反应,制作性早熟模型[40]。此外,Davis 等[41]研究发现雌鼠对幼鼠的护理及自身发育情况随环境条件而改变,当雌性大鼠孕期及产后护理条件较差时,大鼠及其幼崽会产生焦虑进而影响性成熟和性动机发展。此类不良生活条件诱导的性早熟模型可用于探讨家庭环境及压力导致的性早熟研究。

2.6　光照及褪黑素诱导法

选取 21 日龄雌性大鼠暴露于 24 h 持续光照下,持续 7 d 制作光照诱导性早熟模型[42]。Yang 等[43]选取 PND 10 小鼠每日早上腹腔注射

15 mg/kg 褪黑激素成功制备了性早熟模型,褪黑激通过激活垂体中的细胞外调节蛋白激酶(extracellular regulated protein kinases, ERK1/2)信号传导促进 FSH 的合成,从而增加雌激素水平,加速青春期的开始。光照是啮齿类动物内源性节律的触发器,在自然环境下松果体按昼低夜高的节律分泌褪黑素调节生殖轴[44-45]。夜间褪黑素分泌下降可导致性成熟,外源性褪黑素摄入及长期光照均可导致人体夜间褪黑素分泌减少,导致青春期提前[46]。此类模型操作简单,多用于探讨光环境及褪黑素与性早熟的相关性研究。

2.7 锰暴露染毒法

锰暴露染毒制作性早熟模型分为急性染毒与慢性染毒两种方法。慢性染毒选取 12 日龄的雌性大鼠每天灌胃氯化锰($MnCl_2$)(10 mg/kg)[47-48]。急性染毒是在 24 日龄的雌性大鼠的第三脑室植入套管,待大鼠恢复 4 d 后,于 9:00 通过套管往第三脑室中注射 10 μg 的 $MnCl_2$[49]。与急性染毒法相比,慢性染毒操纵简单,更为常用。锰离子是人体正常生理所需的微量元素,研究发现青春期前长期暴露于锰元素会调节下丘脑 GnRH 的特定上游基因,刺激青春期相关激素如 LH、FSH 和 E_2 的血清水平升高,促进青春期提前出现,引起性早熟[50],其可能通过胰岛素样生长因子 1(insulin-like growth factor 1, IGF-1)/蛋白激酶 B(protein kinase B, Akt)/雷帕霉素靶蛋白(mammalian target of rapamycin, mTOR)途径[49]、一氧化氮/环鸟苷单磷酸(cyclic guanosine monophosphate, cGMP)/蛋白激酶 G[51]等途径发挥作用。

2.8 环境内分泌干扰物染毒法

环境内分泌干扰物是一类具有干扰人体生殖、神经和免疫系统等功能的外源性化学物质,主要是由人类的生产和生活中排放到环境中的有机污染物组成,如双酚 A(bisphenol A, BPA)、玉米赤霉烯酮(zearalenone, ZEA)、5-羟甲基糠醛(5-hydroxymethyl furfural, HMF)、邻苯二甲酸酯(phthalic acid ester, PAEs)、阻燃剂等。大量研究发现,儿童长期接触环境内分泌干扰物导致性早熟[52]。运用此类方法制作性早熟模型操作较为简单,造模时间偏长,但部分染毒物研究较少,造模剂量、时间等方面仍需深入研究,此外诱导的性早熟模型是否为真性性早熟模型也有待进一步研究。

2.8.1 BPA 染毒法

取 21 日龄雌性大鼠连续 10 d 给予 0.25 mg/kg BPA 灌胃制备中枢性性早熟模型[53]。BPA 是一种芳香化合物,是生产聚碳酸酯塑料和合成树脂的原材料,常被用于食品和饮料包装、婴儿奶瓶、玩具等日常生活物品中,其分子具有雌激素样和致肥胖作用[54]。BPA 可以通过皮肤、饮食、母乳、胎盘等途径进入人体[55-57]。临床研究发现,特发性中枢性性早熟女童尿液及血液中 BPA 含量明显高于较正常女童,BPA 暴露与学龄女孩患特发性中枢性性早熟(idiopathic central precocious puberty, ICPP)的概率增加有关[58-59]。

2.8.2 双酚 S(Bisphenol S, BPS)染毒法

陈磊等[60]选取 15 日龄的雌性 Wistar 大鼠进行分组,连续 4 周每日分别灌胃 0.2 mg/kg、1 mg/kg、5 mg/kg 双酚 S(bisphenol S, BPS),发现 5 mg/kg BPS 灌胃的雌性大鼠的阴道开口时间明显提前,且下丘脑中 IGF-1、胰岛素样生长因子 1 受体(insulin-like growth factor 1 receptor, IGF-1R)、磷酸肌醇-3-激酶(phosphatidylinositol 3-kinase, PI3K)、Akt、mTOR、GnRH 蛋白表达水平明显上升,成功制备了 BPS 染毒诱导的性早熟模型。BPS 是 BPA 最常用的替代物,二者结构类似,但具有比 BPA 更大的毒性作用[61-62],目前 BPS 对性发育相关研究较少。

2.8.3 PAEs 染毒法

Liu 等[63]选取 15 日龄的雌性大鼠,连续四周灌胃 0、250、500 和 1000 mg/(kg·d)邻苯二甲酸二(2-乙基己)酯(Di 2-ethyl hexyl phthalate, DEHP)制作邻苯二甲酸酯染毒性早熟模型,研究发现,DEHP 可缩短雌性大鼠的阴道开放时间并延长发情周期,1000 mg/(kg·d)组大鼠阴道开放时间、发情周期较其他组相比改变最为明显,其可能影响了下丘脑 GnRH、KISS-1 和 GPR54 的 mRNA 和蛋白表达水平,干扰下丘脑的内分泌调控,导致性早熟[64]。Yu 等[65]选取 15 日龄的雌性大鼠每天灌胃 5 mg/kg DEHP 亦成功制备了性早熟模型。PAEs 是最常用的增塑剂,广泛用于生产个人护理产品、油漆、建筑材料、家居、儿童玩具、清洁材料及食品药品包装材料中,可通过皮肤、饮食、呼吸道吸入进入人体[66]。DEHP 是目前人类应用最广泛、接触最多的一类 PAEs。一项荟萃分析指出,PAEs 中的 DEHP 和邻苯二甲酸二丁酯(Di butyl phthalate, DBP)暴露可能与女孩的性早熟有关[67]。

2.8.4　HMF 染毒法

HMF 是一种有机化合物,是在氨基酸存在下果糖和葡萄糖脱水产生,存在于加热、烘烤、油炸等多种加工食品中[68]。目前关于 HMF 对青春期发育方面的研究较少。Elmaoğulları 等[69]第 1 次报道了 HMF 对青春期发育影响的研究,将 21 日龄雌性大鼠分为 3 组,分别于 9:00 ~ 10:00 灌胃 0、750、1500 mg/(kg·d)的 HMF,每天 1 次,每周 6 d,直到 PND 44 处死,测量相关性发育指标,研究发现高剂量 HMF 组较对照组相比阴道开口更早、促黄体生成素和 E_2 含量更高,证实青春期前期暴露于高剂量 HMF 会导致性早熟。

2.8.5　ZEA 诱导法

Yang 等[70]将 15 日龄雌性大鼠进行分组,连续 5 d 灌胃 0.2、1 和 5 mg/kg ZEA 制备性早熟模型,当 ZEA 染毒剂量达到 5 mg/kg 时大鼠阴道开放时间显著加快、子宫重量明显增加,血清 FSH、LH 和 E_2 水平显著升高,下丘脑 GnRH 的 mRNA 和蛋白表达均明显升高。孙燕等[71]选取 21 日龄的雌性大鼠每日 15:00 灌胃 ZEA 400 mg/kg 1 次,该方法亦成功制备了性早熟模型。ZEA 是由一种类雌激素样真菌毒素,具有拟雌激素样作用,较 BPA 等工业污染物相比,广泛存在于发霉变质的玉米、小麦、大麦、燕麦、牛奶等饮食物中。临床研究发现性早熟儿童外周血中 ZEA 的检出率及含量均显著高于健康儿童[72]。ZEA 诱导中枢性性早熟模型与雌二醇相似,可直接激活 KP 神经元以刺激 GnRH mRNA,其作用机制与 Kisspeptin/GPR54/GnRH 信号通路有关[73]。

2.8.6　其他染毒法

除环境内分泌干扰物以外,阻燃剂、薰衣草油(lavender oil, LO)、乙烯利等物质长期染毒亦可导致性早熟。既往病案报道提示,长期吸入含薰衣草香料的产品会导致儿童的乳房发育[74]。Kim 等[75]选取 18 日龄的雌性大鼠随机分为对照组、室内 LO 环境暴露组、LO 喷鼻组,发现暴露于 LO 的大鼠较对照组相比,阴道开口时间显著提前,促性腺激素和 E_2 水平明显升高。Gouesse 等[76]研究发现大鼠在交配前到幼崽断奶期间持续给予溴化物阻燃剂(bromide flame retardant, BFR)混合物(0、0.06、20 或 60 mg/(kg·d))喂养,组织形态学分析显示,暴露于 0.06 mg/(kg·d) BFR 混合物的二代雌性大鼠的乳腺上皮细胞快速发育,乳腺导管面积、厚度和管腔面积均有增加趋势。徐莹等[77]选取不同浓度乙烯利(134、269、538 mg/kg)自 15 日龄开始每日灌胃至 21 日龄大鼠,证实 269 mg/kg 乙烯利具有促进雌性大鼠阴道开口提前、HPGA 轴相关基因表达增加的作用。Kakeyama 等[78]对怀孕 15 d 的雌性大鼠灌胃 2,3,7,8-四氯二苯并二恶英(2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD)(每周 0、200、800 ng/(kg·d))直至二代雌性大鼠断奶(PND21),暴露于 TCDD 母鼠所生的雌性后代阴道开口和第一次发情比对照组所生的后代大约提前 4 ~ 7 d,卵巢明显增大。

2.9　颅脑辐射诱导法

Roth 发明颅脑辐射诱导性早熟模型,根据大鼠年龄将其分为婴儿期及幼年大鼠组,婴儿期大鼠选取 12 ~ 16 日龄雌性大鼠并分组接受 4、5、6 Gy 的单剂量颅脑照射;幼年大鼠选取 21 ~ 23 日龄的雌性大鼠并分组接受 4、6、9 或 2 × 9 Gy 的单剂量颅脑照射[79]。使用颅骨 ^{60}Co 辐照技术通过背野进行辐射,辐射源距离皮肤 80 cm,根据不同组的剂量要求,照射持续时间 2 min 52 s ~ 6 min 57 s,为了减少辐射对腺体损伤,Roth 等[79]用 5 cm 的铅块对大鼠鼻子、前额和躯干进行遮挡。研究证实,GnRH 脉冲发生器对辐射敏感,婴儿期接触低辐射剂量(5、6 Gy)的颅脑照射会导致青春期加速开始以及 LH、E_2 水平升高,辐射诱导的性早熟可能是由抑制性 γ 氨基丁酸(γ-aminobutyric acid 或 Gamma-aminobutyric acid, GABA)能神经元的损伤引起[80]。该模型造模时间短,但对场地、操作、设备要求较高,适用于放射性物质与性早熟的相关性研究。

2.10　注射诱导法

脑内药物注射诱导法对操作要求较高,目前应用较少,既往有使用 KISS-1 肽、神经肽 Y(neuropeptide Y, NPY)等药物通过脑内注射成功制作性早熟模型。Navarro 等[81]在 25 日龄的雌性大鼠进行颅脑插管,PND 26 ~ 31 通过套管将 KISS-1 肽(1 nmol, q12 h)输送到大脑外侧脑室进行诱导,该方法制备的性早熟模型阴道开口时间较正常组明显提前,子宫重量、血清 LH、E_2 水平均超过正常组。Minami 等[82]使用不同剂量 NPY 通过套管单次注射到 30 日龄雌性大鼠的第 3 脑室,其中 10 ~ 20 μg 的 NPY 可以促进大鼠阴道开放和第 1 次排卵的时间提前,该药物通过激活下丘脑-垂体-性腺轴导致大鼠青春期提前。该方法诱导的性早熟模型在雏鸡中亦得到了较好验证[83]。

表 1 造模方法对比

Table 1 Comparison of modeling methods

名称 Title	动物 Animal	方法 Method	评估指标 Evaluation indicators	优缺点 Advantages and disadvantages	适用 Apply	参考文献 References
达那唑诱导法 Danazol induction method	5 日龄 Wistar 雌性大鼠或清洁级 SD 雌性大鼠 5 day old Wistar female rats or SD female rats of clean grade	单次皮下注射 300 μg 达那唑 Single subcutaneous injection 300 μg Danazol	阴道涂片；阴道开口时间、首次发情时间、正常发情周期时间；子宫和卵巢系数、卵泡发育情况；激素水平；下丘脑 KISS-1、GPR54 和 GnRH 的 mRNA 及 kisspeptin 的表达 Vaginal smear; vaginal opening time, first oestrus time, normal oestrus cycle time; uterine and ovarian coefficients, follicular development; hormone level; expression of KISS-1, GPR54 and GnRH mRNA and kisspeptin in hypothalamus	优点：方法简单、周期短、持续时间长、性周期和正常青春期动物性周期完全一致 Advantages: the method is simple, the cycle is short, the duration is long, and the sexual cycle is completely consistent with that of normal pubertal animals	中枢性性早熟 Central precocious puberty	[18-20]
NMA 诱导法 NMA induction method	26 日龄 SD 雌性大鼠 26 day old SD female rats	每日 14:00 和 16:00 皮下注射 NMA 40 mg/kg NMA 40 mg/kg subcutaneous injection at 14:00 and 16:00 every day	卵巢指数、子宫指数、卵巢黄体出现率、子宫壁厚度；阴道口开放时间、第一个发情间期出现的时间；阴道涂片；GnRH 和 NMDA-R1 基因表达；激素水平 Ovarian index, uterine index, occurrence rate of ovarian corpus luteum, thickness of uterine wall; the opening time of vaginal orifice and the time of the first estrus interval; vaginal smear; GnRH and NMDA-R1 gene expression; hormone level	优点：安全、简便、有效、性发育特征与正常青春启动组的变化相同 Advantages: safe, simple, effective, with the same sexual development characteristics as those in the normal youth initiation group	中枢性性早熟 Central precocious puberty	[21-25]
雌二醇诱导法 Estradiol induction	15 日龄 SPF 级雌性 SD 大鼠 15 day old SPF female SD rats	灌胃给予雌二醇 50 μg/kg，每天 1 次，连续 5 d Estradiol 50 μg/kg by gavage once a day for 5 days	阴道涂片；阴道开口时间、首次发情时间、正常发情周期时间；子宫、卵巢、阴道指数；激素水平；下丘脑 KISS-1、GPR54 和 GnRH 与垂体 GnRH 受体 mRNA Vaginal smear; vaginal opening time, first oestrus time, normal oestrus cycle time; index of uterus, ovary and vagina; hormone level; hypothalamic KISS-1, GPR54, GnRH and pituitary GnRH receptor mRNA	优点：操作较简单、与正常青春启动组的变化相同 Advantages: the operation is simple, and the changes are the same as those in the normal youth initiation group	中枢性性早熟 Central precocious puberty	[26]
高脂饲料造模法 High fat feed modeling method	SD 大鼠或 Wistar 大鼠 SD rats or Wistar rats	断奶后一代鼠持续给予高脂饲料喂养；哺乳期一代鼠及断奶后二代仔鼠高脂饲料；肥胖型性早熟一代鼠全程及二代鼠断奶后给予高脂饲料喂养 After weaning, the rats were fed with high-fat diet continuously; High fat diet for the first generation of lactating rats and the second generation of weaned rats; The first generation of obese precocious puberty rats were fed with high-fat diet during the whole process and after weaning in the second generation	阴道涂片；下丘脑 GnRH mRNA、KISS-1 mRNA、GPR54 mRNA、垂体 GnRH-R mRNA 的表达；激素水平；阴道口开放时间、第一个发情间期出现的时间；子宫、卵巢指数 Vaginal smear; expression of GnRH mRNA, KISS-1 mRNA, GPR54 mRNA in hypothalamus and GnRH-R mRNA in pituitary; hormone level; the openingtime of vaginal orifice and the time of the first estrus interval; index of uterus and ovary	优点：操作简单、与正常青春启动组的变化相同 缺点：高脂饲料配方无统一标准；二代鼠繁殖操作较为困难，造模时间较长 Advantages: simple operation, same changes as normal youth initiation group Disadvantages: There is no uniform standard for high-fat feed formula; It is difficult to reproduce the second generation mice, and the modeling time is long	肥胖型中枢性性早熟 Obese central precocious puberty	[28,33]

续表1

名称 Title	动物 Animal	方法 Method	评估指标 Evaluation indicators	优缺点 Advantages and disadvantages	适用 Apply	参考文献 References
不良生活条件造模法 Modeling of adverse living conditions	SD 大鼠 SD rats	从 P2 ~ 10 暴露于低资源环境(西方饮食/限制筑巢资源)或在幼崽 P2 ~ 14 期间与母亲分离 Exposure to low safety (Western diet/limited nesting materials) from P2 ~ 10 or separation from mother during P2~14 of the young	阴道开放时间;激素水平;下丘脑基因表达 Vaginal opening time; hormone level; hypothalamus gene expression	缺点:操作及场地要求较高 Disadvantages: high requirements for operation and site	家庭环境及压力导致的性早熟研究 Study on precocious puberty caused by family environment and stress	[39-40]
光照及褪黑素诱导法 Light and melatonin induction method	21 日龄雌性 SD 大鼠或产后 10 日龄的雌性 KM 菌株小鼠 21 day old female SD rats or 10 day postpartum female KM strain mice	24 h/d 持续光照,光照强度(300 ± 20) Lux 或每天 8:00 腹腔注射 15 mg/kg 褪黑素 24 h/d continuous light, light intensity (300 ± 20) Lux or intraperitoneal injection of 15 mg/kg melatonin at 8:00 every day	激素水平;褪黑素水平;阴门开启及首个动情间期时间;阴道涂片;子宫、卵巢脏器系数;松果体 Hormone level; melatonin level; the time of vulva opening and the first estrus; vaginal smear; uterine and ovarian organ coefficients; pineal gland	优点:操作简单 Advantages: simple operation	探讨光环境及褪黑素与性早熟的关系 Study on the relationship between light environment, melatonin and precocious puberty	[42-43]
锰暴露染毒法 Manganese exposure induction method	急性染毒:24 日龄的 SD 雌性大鼠;慢性染毒:12 日龄的 SD 雌性大鼠 Acute exposure: 24 day old SD female rats; Chronic exposure: 12 day old SD female rats	急性染毒:第三脑室植入套管,待大鼠恢复 4 d 后,于 9:00 通过套管往第三脑室中注射 10 μg 的 $MnCl_2$;慢性染毒:每天灌胃 $MnCl_2$ (10 mg/kg) Acute exposure: cannula was implanted into the third ventricle. After 4 days of recovery, 10 μg $MnCl_2$ was injected into the third ventricle through the cannula at 9:00; Chronic exposure: $MnCl_2$ (10 mg/kg) by gavage every day	激素水平;阴道开口时间;下丘脑及垂体 mRNA 及蛋白测定 Hormone level; vaginal opening time; determination of mRNA and protein in hypothalamus and pituitary	慢性染毒:优点 操作简单,缺点 造模时间较急性染毒长;急性染毒:优点 造模时间短,缺点 操作复杂 Chronic poisoning: advantages: simple operation, disadvantages: modeling time is longer than acute poisoning. Acute poisoning: advantages: short modeling time, disadvantages: complex operation	探讨暴露于环境金属 Mn 与性早熟的研究 Study on the relationship between environmental metal Mn exposure and precocious puberty	[47-49]
双酚 A 染毒法 Bisphenol A exposure method	21 日龄雌性大鼠 21 day old female rats	连续 10 d 给予 0.25 mg/kgBPA 灌胃 0.25 mg/kg BPA was administered by gavage for 10 consecutive days	下丘脑、卵巢及子宫脏器系数;激素水平;下丘脑 KISS-1、GPR54 基因表达 Organ coefficients of hypothalamus, ovary and uterus; Hormone level; KISS-1 and GPR54 gene expression in hypothalamus	优点:操作简单,与正常青春启动的变化相同 Advantages: simple operation, same changes as normal youth startup		[53]
双酚 S 染毒法 Bisphenol S poisoning method	15 日龄雌性 Wistar 大鼠 15 day old female Wistar rats	连续 4 周每日灌胃 5 mg/kg BPS Daily intragastric administration of 5 mg/kg BPS for 4 consecutive weeks	阴道开口时间;下丘脑组织病理变化;下丘脑中 IGF-1、GnRH mRNA 表达水平 Vaginal opening time; histopathological changes of hypothalamus; expression of IGF-1 and GnRH mRNA in hypothalamus	优点:操作简单;缺点:造模时间较长 Advantages: simple operation; Disadvantages: long molding time		[60]

续表1

名称 Title	动物 Animal	方法 Method	评估指标 Evaluation indicators	优缺点 Advantages and disadvantages	适用 Apply	参考文献 References
邻苯二甲酸酯染毒法 DEHP poisoning method	15 日龄的雌性 Wistar 大鼠或 SD 大鼠 15 day old female Wistar rats or SD rats	连续 4 周灌胃 1000 mg/kg 或 5 mg/kg DEHP 1000 mg/kg or 5 mg/kg DEHP by gavage for 4 consecutive weeks	子宫卵巢系数;下丘脑 GnRH 基因及蛋白水平;激素水平;阴道涂片 Coefficient of uterus and ovary; GnRH gene and protein level in hypothalamus; hormone level; vaginal smear	优点:操作简单;缺点:造模时间较长,剂量无统一标准 Advantages: simple operation; Disadvantages: long modeling time, no unified standard for dosage	环境内分泌干扰物与性早熟研究 Study on environmental endocrine disruptors and precocious puberty	[63-65]
5-羟甲基糠醛染毒法 6-5-hydroxymethylfurfural exposure method	21 日龄雌性 Wistar 大鼠 21 day old female Wistar rats	自 P21 ~ 44 于 9:00 ~ 10:00 之间灌胃 1500 mg/(kg·d)的 HMF,每天 1 次,每周 6 d 1500 mg/(kg·d) HMF was administered by gavage from P21 ~ 44 at 9:00 to 10:00, once a day, 6 days a week	阴道开口时间;子宫、卵巢大小及卵泡评估;激素水平 Vaginal opening time; evaluation of uterus, ovary size and follicle; hormone level	优点:操作简单 Advantages: simple operation		[69]
玉米赤霉烯酮诱导法 Zearalenone induction method	15 日龄雌性 SD 大鼠或 21 日龄雌性 SD 大鼠 15 day old female SD rats or 21 day old female SD rats	连续 5 d 灌胃 5 mg/kg ZEA 或每日 15:00 灌胃 ZEA 400 mg/kg 至阴道开口 5 mg/kg ZEA by gavage for 5 consecutive days or 400 mg/kg ZEA by gavage to the vaginal opening at 15: 00 every day	阴道开口时间、第 1 发情间期出现,性腺及性器官检查;阴道涂片、激素水平、下丘脑基因及蛋白表达 The time of vaginal opening and the first estrus interval appeared, examination of gonads and sexual organs; vaginal smear, hormone level, gene and protein expression in hypothalamus	优点:操作简单;缺点:动物造模年龄、剂量、时间均无统一标准 Advantages: simple operation; Disadvantages: there is no uniform standard for age, dose and time of animal modeling		[70-71]
LO 染毒法 LO poisoning method	15 日龄雌性 SD 大鼠 15 day old female SD rats	分别予 LO 扩散器在室内暴露及鼻喷雾每天 1 次(剂量范围为 72 ~ 125 μL)处理至阴道开口时 When exposed indoors with LO diffuser and treated to vaginal opening with nasal spray once a day (dose range: 72 ~ 125 μL)	阴道开口时间;激素水平;卵巢重量测量 Vaginal opening time; hormone level; ovarian weight measurement	优点:操作较简单;缺点:设备要求较复杂 Advantages: simple operation; Disadvantages: complicated equipment requirements		[75]
BFR 混合物染毒法 BFR mixture poisoning method	SD 大鼠 SD rats	交配前到幼崽断奶期间持续给予 BFR 混合物(0.06 mg/(kg·d))喂养 BFR mixture (0.06 mg/(kg·d)) was administered continuously before mating and during weaning	乳腺切片观察 Observation of breast section	缺点:二代鼠繁殖操作较复杂;仅监测乳腺情况,未监测其他指标 Disadvantages: The reproduction of the second generation mice is more complicated; only monitor the mammary gland, not other indicators		[76]
乙烯利染毒法 Ethephon exposure method	15 日龄 SPF 级 SD 雌性大鼠 15 day old SPF SD female rats	自 15 日龄开始每日灌胃 269 mg/kg 乙烯利至 21 日龄 From the age of 15 days to the age of 21 days, 269 mg/kg ethephon was orally administered daily	阴道开口时间;激素水平;下丘脑及垂体基因检测 Vaginal opening time; hormone level; gene detection of hypothalamus and pituitary	优点:操作简单;缺点:实验取材时间点设计不够完整,因此未能观察到激素水平的变化 Advantages: simple operation; Disadvantages: the design of the time point for the experiment was not complete enough, so the change of hormone level was not observed		[77]

续表1

名称 Title	动物 Animal	方法 Method	评估指标 Evaluation indicators	优缺点 Advantages and disadvantages	适用 Apply	参考文献 References
TCDD 染毒法 TCDD exposure method	LE 雌性大鼠 LE female rats	怀孕 15 d 的雌性大鼠灌胃 TCDD(每周 0、200、800 ng/(kg·d))直至二代雌性大鼠断奶(PND21) TCDD (0, 200, 800 ng/(kg·d) per week) was administered to female rats at the 15th day of pregnancy until weaning of the second generation female rats (PND21)	阴道开口时间;第一次发情、第一次排卵和发情周期性;卵巢重量及切片观察;卵巢代偿性肥大测试 Vaginal opening time; the first oestrus, the first ovulation and the cycle of oestrus; ovarian weight and section observation; ovarian compensatory hypertrophy test	优点:造模操作简单;缺点:卵巢代偿性肥大测试较复杂 Advantages: simple modeling operation; Disadvantages: the test of ovarian compensatory hypertrophy is complex		[78]
颅脑辐射诱导法 Brain radiation induction method	SD 雌性大鼠 SD female rats	12 ~ 16 日龄雌性大鼠接受 5、6 Gy 的单剂量颅脑照射,使用颅骨 ^{60}Co 辐照技术通过背野进行辐射,辐射源皮肤距离为 80 cm,并用 5 cm 的铅块对鼻子、前额和躯干进行遮挡 12 ~ 16 day old female rats received 5 and 6 Gy single dose brain irradiation. They used ^{60}Co skull irradiation technology to radiate through the back field. The distance of the radiation source skin was 80 cm, and a 5 cm lead block was used to shield the nose, forehead and trunk	阴道开口时间;激素水平 Vaginal opening time; hormone level	优点:造模时间短;缺点:设备要求较高 Advantages: short molding time; Disadvantages: high equipment requirements	放射性物质与性早熟 Radioactive substances and precocious puberty	[79-80]
KISS-1 肽脑内注射 Intracerebral injection of KISS-1 peptide	25 日龄 Wistar 雌性大鼠 25 day old female Wistar rats	在产后第 25 天用静脉导管植入,套管降低到颅骨表面以下 3 mm 的深度;插入点为后部 1 mm,后向后插入点为 1.2 mm。在产后 26 ~ 31 d 之间通过套管将 KISS-1 肽(1 nmol, q12h)输送到大脑外侧脑室 On the 25th day after delivery, intravenous catheters were implanted, and the cannula was lowered to a depth of 3 mm below the surface of the skull; The insertion point is 1 mm at the rear and 1.2 mm at the rear. KISS-1 peptide (1 nmol, q12h) was delivered to the lateral cerebral ventricle via cannula between 26 and 31 days postpartum	阴道开口时间;激素水平;子宫重量 Vaginal opening time; hormone level; uterine weight	优点:造模时间短;缺点:操作复杂 Advantages: short molding time; Disadvantages: complex operation	性早熟 Pubertas praecox	[81]
NPY 肽脑内注射 Intracerebral injection of NPY peptide	30 日龄雌性 SD 大鼠 30 day old female SD rats	用 10 ~ 20 μg 的剂量范围 NPY 通过套管单次注射到 30 日龄雌性大鼠的第 3 脑室 NPY was injected into the third ventricle of 30 day old female rats through cannula in a single dose range of 10 ~ 20 μg	阴道开口时间、首次排卵时间;激素水平 Time of vaginal opening and first ovulation; hormone level	优点:造模时间短;缺点:操作复杂 Advantages: short molding time; Disadvantages: complex operation	性早熟 Pubertas praecox	[82]

3 评估指标

3.1 阴道开口时间

阴道开口时间和第 1 次排卵时间通常是青春期开始的决定性标志。初次发情排卵一般在大鼠阴道开口前后,因此阴道开口作为一个无创、易于观察的可靠外部指标,成为研究大鼠性成熟的一个重要检测指标。性早熟实验多选择于 8:00 观察并记录大鼠阴道开口情况。

3.2 阴道涂片及动情周期观察

阴道黏膜上皮受卵巢内分泌功能直接影响,对雌激素十分敏感,啮齿类动物在动情周期不同阶段,阴道黏膜会根据体内激素水平变化出现较为典型的改变[84]。根据阴道脱落细胞涂片的观察可方便准确地了解卵巢功能。成年雌性大鼠受性激素影响,规律出现动情前期、动情期、动情间期和动情后期 4 个阶段,阴道涂片有明显的周期性变化。动情间期阴道涂片以白细胞为主;动情前期以有核上皮细胞为主;动情期以角质化的上皮细胞为主;动情后期角质化上皮细胞、有核上皮细胞和白细胞 3 种细胞比例相当[85]。因阴道涂片中细胞形态差异较大,形态辨认简单,操作简便,是目前常用的大鼠性周期判定方法。

3.3 血清性激素水平测定

血清性激素检测是临床儿童性早熟诊疗的常用检测指标,主要包括 LH、FSH、E_2。临床中可根据血清性激素水平来判断性早熟类型,当儿童 LH > 3.0 ~ 5.0 U/L 可肯定已有中枢性青春发动[86-87],当 E_2> 367 pmol/L 时需要高度警惕卵巢囊肿或肿瘤可能[88]。LH 和 FSH 受垂体前叶 GnRH 刺激而产生,广泛分布于外周血液循环中,大鼠性成熟过程中激素分泌情况大体与人类相似,因此检测外周血中 LH 和 FSH 的浓度是判定性成熟的有效途径。

3.4 下丘脑基因检测

中枢性性早熟是由下丘脑-垂体-性腺轴功能提前启动引起第二性征提前发育。下丘脑分泌的 GnRH 在生殖发育中起关键性的作用,呈脉冲式分泌,在婴儿期处于低水平,随个体的生长发育,GnRH 释放的脉冲幅度及频率不断增加,刺激性激素分泌增加[89],因此检测 GnRH mRNA 的表达情况、免疫组化法鉴定下丘脑 GnRH 的生成可作为判定 HPGA 功能激活的重要指标。临床研究发现性早熟与遗传学因素存在相关性。儿童与家庭成员青春期开始的年龄具有强相关性[90-91]。性早熟的家系中发现基因突变是导致青春期启动的重要因素,如 KISS1、KISS1R、MKRN3 及 DLK1 基因等[92-93]。此外,RFRP3/GPR147[94]、Lin28/Let7[95]、GPR54/GnRH[96]、IGF-1/PI3K/Akt/mTOR[97]、Kisspeptin/GPR54[98]等多种信号通路通过调节大鼠的青春期发育和性成熟来参与性早熟的发病机制。因此检测下丘脑基因指标水平有利于研究性早熟的发病机制及药物治疗途径。

3.5 性器官检测

性器官检测包括子宫、卵巢指数,卵巢子宫切片观察、子宫厚度测定、卵巢黄体情况等。在生长发育过程中,促性腺激素刺激性器官成熟并分泌卵巢激素,故临床诊断中,当女童盆腔 B 超显示子宫、卵巢容积增大,且卵巢内可见多个直径 > 4 mm 卵泡时提示青春期发育。研究发现性早熟女童子宫和卵巢各项指标均较正常儿童明显改变,B 超检查可在无创伤前提下对患儿性器官的形态、大小,子宫内膜的厚度以及卵泡的数量和体积等信息进行清晰、完整、准确的显现及测量,并能对体内 HPGA 水平的活跃度进行评估,评判性早熟的真假性质,临床应用十分广泛[99-100]。实验动物在性发育前后性器官表现存在差异,性发育后会出现子宫和卵巢增大以及卵泡发育等特征,对实验动物进行性器官大小计算、组织切片观察黄体出现情况等方法可直观体现发育情况。因此,在性早熟动物实验中对实验动物性器官组织学观察及测量是必不可少的。

4 结语

儿童性早熟发病率逐年升高,性早熟动物模型可以为研究性早熟的发病机制和治疗提供有价值的信息。目前性早熟造模动物选择、造模方法及观察指标众多,达那唑诱导法及 NMA 诱导法为性早熟造模常用方法。在性早熟研究时,应根据研究内容、各造模方法的适用条件及优缺点,选择适合的造模方法。因此深入全面了解性早熟动物造模的方法及相关评估指标有利于研究各种易感因素导致儿童性早熟的发病机制,并可对药物治疗作用进行效果评估,深入挖掘药物的治疗机制。

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