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草鱼不同月龄性腺组织学观察及性别特征基因 *cyp19a1a* 和 *amh* 的表达分析*

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摘要 为探讨草鱼(*Ctenopharyngodon idella*)性腺发生、性别分化及发育规律,本研究对1、2、3、4、5、6、12、24、36和48月龄草鱼性腺组织结构以及性别特征基因 *cyp19a1a* 和 *amh* 的表达差异进行了分析。组织切片结果显示,2月龄时,首次在生殖嵴中观察到原始生殖细胞,标志原始性腺形成。3月龄时,雌性草鱼性腺中观察到卵巢腔和卵巢小叶。4月龄时,观察到卵原细胞,表明其在3月龄时出现解剖学分化,4月龄时出现细胞学分化。4月龄雄性草鱼在性腺中观察到输精导管,5月龄时观察到精原细胞,表明其在4月龄时出现解剖学分化,5月龄时出现细胞学分化。12、24、36和48月龄草鱼卵巢分别处于发育的第I、II、III和IV期,精巢则分别为发育的第II、II、III和IV期。荧光定量结果显示,雌性特征基因 *cyp19a1a* 在卵巢中的表达量总体呈先上升后下降再上升的趋势,2月龄时显著上调($P<0.05$),3、6和48月龄时处于峰值。雄性特征基因 *amh* 在精巢中表达量总体呈先上升后下降的趋势。2月龄时显著上调($P<0.05$),5月龄时达到峰值。综上,草鱼性腺发育启动时间约为2月龄,雌雄性腺分化时间分别约为3月龄和4月龄,至4月龄时雌雄性腺发育成熟。本研究结果丰富了草鱼的繁殖生理学资料,也为其性别调控技术研究奠定了基础。

关键词 草鱼; 性别分化; 组织切片; 基因表达

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生殖是鱼类繁育后代的核心过程,鱼类繁殖的前提是其性腺正常发育。性腺发育是指原始生殖细胞起源至性腺发育成熟的整个阶段,可细分为发生、分化和成熟3个阶段。原始生殖细胞迁移至生殖嵴后与周围体细胞共同组成原始性腺,经过发育后进行分化,

再进一步发育至成熟。鱼类性腺分化方式主要有雌雄异体、雌雄同体和兼性3种类型(Wang *et al.*, 2023),其中,雌雄异体是硬骨鱼类最常见的性腺分化方式。雌雄异体又可分为分化型和未分化型两种,其中,分化型是指原始性腺直接分化为精巢或卵巢,硬骨鱼类

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中大多数为分化型,如鲤鱼(*Cyprinus carpio*)(Jiang *et al*, 2020)、大黄鱼(*Larimichthys crocea*)(游秀容等, 2012)、黄颡鱼(*Pelteobagrus fulvidraco*)(彭丽娜等, 2014)等。开展性腺分化和发育研究不仅对鱼类繁殖性能调控、品种资源开发利用等具有重要意义,还可以为单性诱导提供科学依据。

已有研究表明,与性别相关的基因在鱼类性别决定、性腺分化以及生殖和发育等生物学过程中发挥重要作用(Fajkowska *et al*, 2020)。在多数分化型硬骨鱼类中, *dmrt1*、*sox9*、*foxl2*、*amh*、*cyp19a1a* 等被认为是参与性别调控的关键基因(李永婧等, 2017)。芳香化酶基因(cytochrome P450 family 19 subfamily A, *cyp19a1a*)通过芳香化酶以芳香化作用使雄激素向雌激素转化,在硬骨鱼类性腺分化和卵巢发育过程中发挥重要作用(Yan *et al*, 2019; 王金等, 2014)。该基因在性腺中特异表达(Sudhakumari *et al*, 2005), 被作为性别向雌性分化的特征基因(Blázquez *et al*, 2008)。抗缪勒氏管激素(anti-Müllerian hormone, *amh*)对于生殖器官结构和生殖细胞的正常分化及发育具有调节作用(Kang *et al*, 2023)。在虹鳟(*Oncorhynchus mykiss*)(Yano *et al*, 2012)、鲤鱼(Wang *et al*, 2023)、草鱼(*Ctenopharyngodon idella*)(姚汶励等, 2019)等鱼类中, *amh* 基因在精巢中的表达量显著高于卵巢,常作为性别向雄性分化的特征基因(Yano *et al*, 2012)。

草鱼与青鱼、鲢鱼、鳙鱼并称为四大家鱼。我国草鱼养殖年产量在养殖鱼类中长期占居首位,2022年养殖产量达到 590.5 万 t,占淡水鱼类养殖总产量的 24.3%(农业农村部渔业渔政管理局等, 2023)。草鱼性腺发育速度受季节、水温、生长环境及地域等多个因素影响,如华北地区草鱼性成熟一般需要 5 年,华南地区 4 年即可性成熟。本研究以华南地区草鱼为研究对象,通过组织切片观察草鱼不同月龄性腺结构变化和后期发育状态,同时,对性腺组织中性别特征基因 *cyp19a1a* 和 *amh* 的表达量进行分析,以期了解草鱼性腺分化时间和发育过程,为草鱼的繁殖生物学和激素诱导草鱼性别逆转等研究积累基础资料。

1 材料与方法

1.1 实验鱼养殖及样品采集

实验用草鱼均来自中国水产科学研究院珠江水产研究所三水基地,从养殖池塘中挑选 300 尾 10 日龄[(1.24±0.63) g]草鱼幼苗放入面积为 12 m²的网箱中养殖,饲养期间不间断充气增氧,每天于 08:00 和 17:00

左右各投喂 1 次,每次投喂量为鱼体重的 2%~3%。实验所用饲料购自广东省佛山市联大农牧科技有限公司。

在实验草鱼为 1、2、3、4、5 和 6 月龄时分别从中随机挑选 20 尾,剪取尾鳍保存在无水乙醇中,用于遗传性别鉴定。另采取每尾鱼性腺组织,一部分连着腹腔膜一起放入 4%的多聚甲醛溶液中固定 24 h,随后保存在 70%的无水乙醇中,用于制作组织切片观察性腺发育情况,剩余性腺组织放入 RNA 保存液(聚合美生物科技有限公司,北京),再转入-80℃保存,用于提取总 RNA 和检测基因表达量。

从试验基地草鱼成鱼养殖池中随机挑选 12、24、36、48 月龄草鱼各 20 尾,剪取尾鳍保存至无水乙醇中,采集性腺组织用于组织切片观察和基因表达分析,方法同上。

1.2 性腺组织切片观察

取实验鱼性腺组织后置于 4%的多聚甲醛中固定 24 h 后,经 75%、85%、90%和 95%酒精分别脱水 4、2、2 和 1 h,再使用无水乙醇脱水 2 次,每次 30 min。脱水完成后,使用 2 组二甲苯分别透明 5~10 min 后,将组织用石蜡包埋后切片,切片厚度为 5 μm,再使用苏木精-伊红染色封片并使用 Zeiss Axio Scope.A1 显微镜观察拍照。

1.3 DNA 提取及实验鱼遗传性别确定

按照基因组 DNA 提取试剂盒(天根,北京)说明书提取基因组 DNA,采用 1%的琼脂糖凝胶电泳检测 DNA 质量。利用 Wang 等(2015)开发的草鱼雌雄特异性分子标记进行 PCR 扩增(F: AGCAGCAGGTAGC GGAAGAG, R: AATAACGACAGTTGACAGGATTG AATG)。PCR 扩增体系包括 Premix Taq™ 10 μL, DEPC 水 6 μL, 性别鉴定正反向引物各 1 μL, cDNA 2 μL, 总体系 20 μL。反应程序: 95℃预变性 4 min; 95℃变性 30 s, 56℃退火 30 s, 72℃延伸 30 s, 共 35 个循环; 72℃延伸 5 min。根据扩增条带有无确定实验鱼的遗传性别为雄或雌。

1.4 RNA 提取及雌雄基因表达分析

使用 RNA 提取试剂盒(北京聚合美生物科技有限公司,北京)提取性腺总 RNA,采用 1%的琼脂糖凝胶电泳检测所提取的总 RNA 质量,使用酶标仪(Biotek Cytation 5)检测其浓度。使用 ToloScript All-in-one RT EasyMix 试剂盒(安徽吐露港生物科技有限公司)将 1 μg 总 RNA 逆转录为 cDNA。逆转录体

系为 20 μ L, 反应条件: 50 $^{\circ}$ C 5 min, 85 $^{\circ}$ C 5 s。cDNA 稀释至 20%后, 使用 2 \times Q3 SYBR qPCR Master mix (ToloBio)试剂和荧光定量仪(ABI QuantStudio6)进行实时荧光定量检测, 该反应体系包括 10 μ L SYBR qPCR Master mix, 正反向引物各 0.5 μ L, ddH₂O 7 μ L, cDNA 2 μ L, 总体积 20 μ L。qPCR 程序: 预变性 95 $^{\circ}$ C 30 s, 循环反应包括 95 $^{\circ}$ C 10 s, 60 $^{\circ}$ C 30 s, 共 40 个循环。以 β -actin 作为内参基因, 采用 $2^{-\Delta\Delta C_t}$ 方法计算定量结果。所用 β -actin、*amh* 和 *cyp19a1a* 基因引物序列来自姚汶励等(2019) (表 1), 引物均由生工生物工程(上海)股份有限公司合成。

表 1 荧光定量引物序列
Tab.1 Primer for real-time PCR

基因 Gene	引物 Primer
<i>amh</i>	F: GACACCATTAGCCGAGTTG
	R: CATGCCTTGGGAATTTGA
<i>cyp19a1a</i>	F: ACACCTGGCAGACAGTATTA
	R: CGTGATGGCGTCTGTAAC
β -actin	F: GATGATGAAATTGCCGCACTG
	R: ACCGACCATGACGCCCTGATGT

1.5 数据处理

所有实验数据均以平均值 \pm 标准差(Mean \pm SD)表示, 数据通过 SPSS 进行单因素方差分析(one-way ANOVA), Duncan's 进行多重比较分析组间差异, $P < 0.05$ 表示差异显著。

2 结果

2.1 不同月龄草鱼卵巢组织学观察

不同月龄草鱼卵巢组织切片结果见图 1。1 月龄草鱼出现生殖嵴(图 1A)。2 月龄时, 生殖嵴中出现原始生殖细胞(图 1B)。3 月龄时, 卵巢腔和卵巢小叶已形成(图 1C)。4 月龄时, 卵巢中出现卵原细胞(图 1D)。5 月龄时, 卵巢继续发育和变大(图 1E)。6 月龄时, 性腺中卵原细胞数量增多(图 1F), 此时卵巢已完全分化。依据刘筠(1993)的卵巢分期标准, 12 月龄草鱼卵巢处于发育 I 期, 卵巢中存在发育变大的卵原细胞和第 II 时相初期的卵母细胞, 其体积均比周围体细胞大, 细胞内核仁清晰可见, 分布在仁膜内缘(图 1G)。24 月龄草鱼卵巢处于发育 II 期, 以第 II 时相初期细胞为主, 也存在第 III 时相初期卵母细胞, 由于细胞间相互挤压, 形状不规则, 细胞质中已积累少量卵黄物质(图 1H)。36 月龄草鱼卵巢以次级卵母细胞为主, 卵

黄颗粒清晰可见, 此时卵巢处于发育第 III 期(图 1I)。48 月龄草鱼卵巢肥大充盈, 内部的卵粒肉眼可见, 卵母细胞减少, 卵黄颗粒增多, 处于发育第 IV 期(图 1J)。

2.2 不同月龄草鱼精巢组织学观察

草鱼不同月龄精巢组织切片结果如图 2 所示。雄性草鱼在 1~3 月龄时均未观察到性腺分化的形态学特征(图 2A~C)。4 月龄时, 草鱼输精导管形成(图 2D)。5 月龄时, 精巢发育变大, 出现精原细胞(Sg)及由精原细胞和结缔组织组成的精小囊(图 2E)。6 月龄时, 精原细胞数量增多, 可以观察到极少量由精原细胞向初级精母细胞过渡的细胞(图 2F)。依据刘筠(1993)的精巢分期标准, 1 龄内草鱼精巢处于发育第 I 期。12 月龄和 24 月龄草鱼精巢发育均处于第 II 期, 二者精巢中均存在精原细胞和初级精母细胞, 且 12 月龄草鱼精巢中以精原细胞为主(图 2G), 24 月龄草鱼精巢中初级精母细胞较多(图 2H)。36 月龄草鱼精巢处于发育第 III 期, 在精小囊中可以观察到少量的由次级精母细胞分裂发育而成的精子细胞(图 2I)。48 月龄的精巢已达到发育第 IV 期, 精子细胞明显增多, 精小囊破裂, 小叶腔中出现成熟精子(图 2J)。

2.3 不同月龄草鱼性腺中雌性特征基因 *cyp19a1a* 的表达量分析

Cyp19a1a 在草鱼卵巢中的表达量检测结果如图 3 所示。2 月龄时 *cyp19a1a* 基因表达量开始显著上调 ($P < 0.05$), 3 月龄时达到第一个峰值, 4 月龄时该基因表达量有所下降, 5 月龄时再次显著上调 ($P < 0.05$), 6 月龄时再次达到峰值。12 月龄和 24 月龄时其表达量有所下降, 36 月龄时再次显著上调 ($P < 0.05$), 48 月龄时该基因表达量再次达到峰值。2~48 月龄 *cyp19a1a* 基因在草鱼卵巢中的表达量均显著高于同期精巢 ($P < 0.05$)。

2.4 不同月龄草鱼性腺中雄性特征基因 *amh* 的表达量分析

amh 在草鱼精巢中的表达量如图 4 所示, 其随月龄增长总体呈现先上升后下降的趋势。2 月龄时 *amh* 基因表达量开始显著上调 ($P < 0.05$), 5 月龄时达到峰值; 6 月龄时该基因表达量有所下降, 但和 5 月龄时无显著差异 ($P > 0.05$)。12~48 月龄基因表达量和 2 月龄表达量相接近。2~48 月龄, *amh* 基因在草鱼精巢中的表达量显著高于同时期卵巢 ($P < 0.05$)。

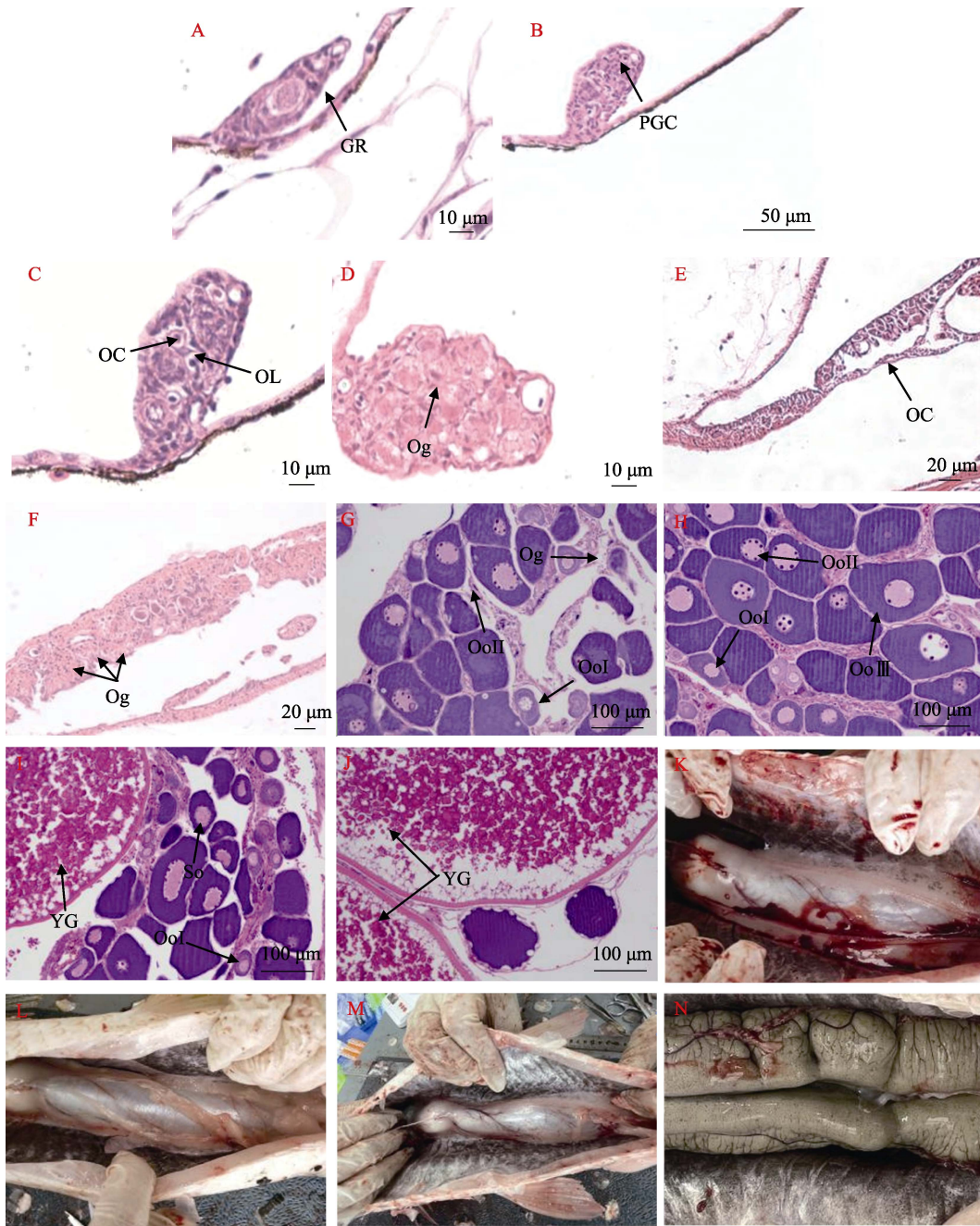


图1 草鱼不同时期卵巢结构观察

Fig.1 Observation of ovarian structure in grass carp at different stages

A: 1月龄草鱼卵巢切片; B: 2月龄草鱼卵巢切片; C: 3月龄草鱼卵巢切片; D: 4月龄草鱼卵巢切片; E: 5月龄草鱼卵巢切片; F: 6月龄草鱼卵巢切片; G: 12月龄草鱼卵巢切片; H: 24月龄草鱼卵巢切片; I: 36月龄草鱼卵巢切片; J: 48月龄草鱼卵巢切片; K: 12月龄草鱼卵巢; L: 24月龄草鱼卵巢; M: 36月龄草鱼卵巢; N: 48月龄草鱼卵巢。

GR: 生殖嵴; PGC: 原始生殖细胞; OC: 卵巢腔; OL: 卵巢小叶; Og: 卵原细胞; Oo I: 第I时相卵母细胞; Oo II: 第II时相卵母细胞; Oo III: 第III时相卵母细胞; SO: 次级卵母细胞; YG: 卵黄颗粒。

A: Ovarian slice of 1-month-old grass carp; B: Ovarian slice of 2-month-old grass carp; C: Ovarian slice of 3-month-old grass carp; D: Ovarian slice of 4-month-old grass carp; E: Ovarian slice of 5-month-old grass carp; F: Ovarian slice of 6-month-old grass carp; G: Ovarian slice of 12-month-old grass carp; H: Ovarian slice of 24-month-old grass carp; I: Ovarian slice of 36-month-old grass carp; J: Ovarian slice of 48-month-old grass carp; K: Ovaries of 12-month-old grass carp; L: Ovaries of 24-month-old grass carp; M: Ovaries of 36-month-old-grass carp; N: Ovaries of 48-month-old grass carp.
GR: Genital ridge; PGC: Primitive germ cells; OC: Ovarian cavity; OL: Ovarian lobules; Og: Oogonia; Oo I: Phase I oocytes; Oo II: Phase II oocytes; Oo III: Phase III oocytes; SO: Secondary oocytes; YG: Yolk granules.

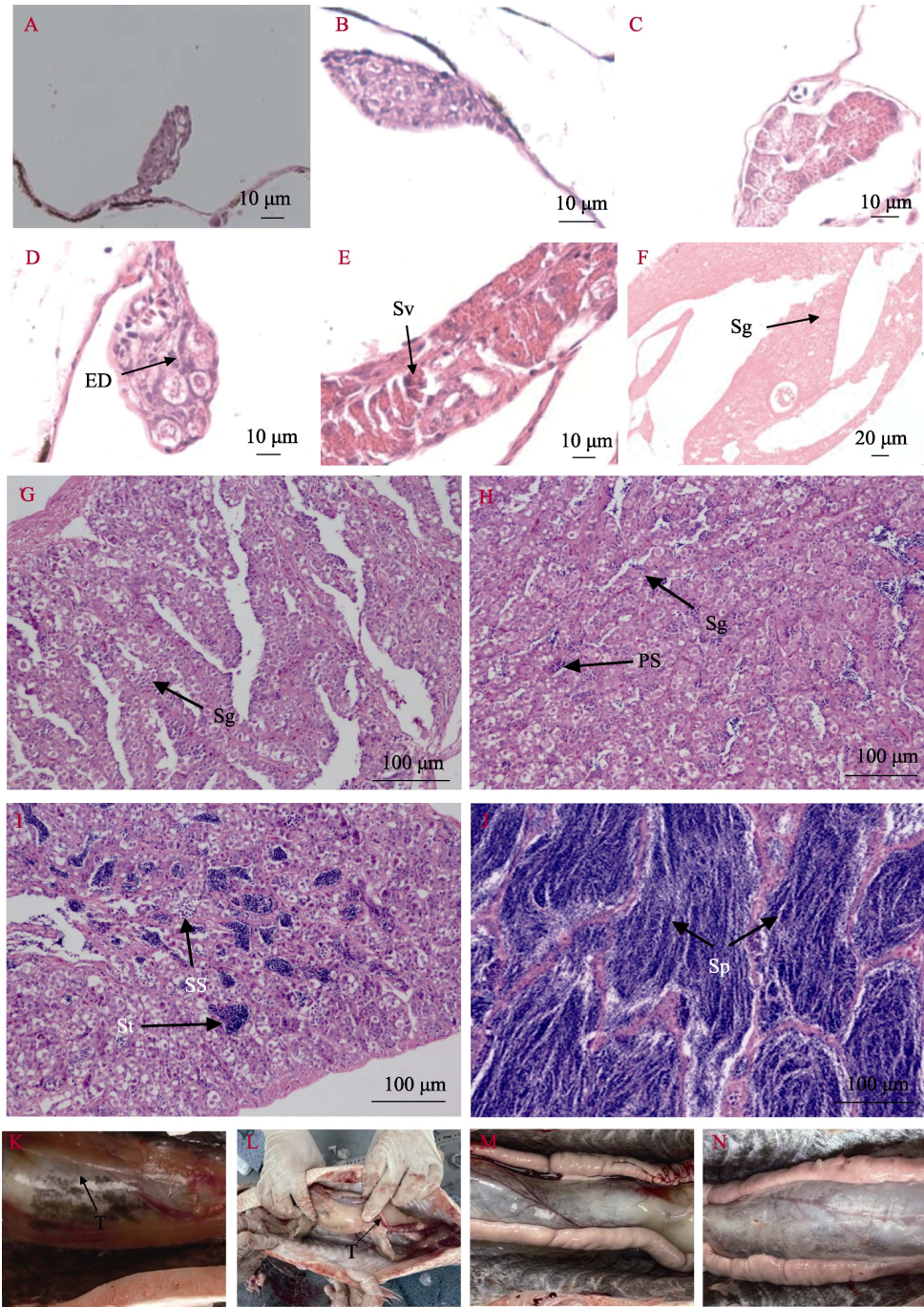


图 2 草鱼不同时期精巢结构观察

Fig.2 Observation of the testicular structure of grass carp at different stages

A: 1 月龄草鱼精巢切片; B: 2 月龄草鱼精巢切片; C: 3 月龄草鱼精巢切片; D: 4 月龄草鱼精巢切片; E: 5 月龄草鱼精巢切片; F: 6 月龄草鱼精巢切片; G: 12 月龄草鱼精巢切片; H: 24 月龄草鱼精巢切片; I: 36 月龄草鱼精巢切片; J: 48 月龄草鱼精巢切片; K: 12 月龄草鱼精巢; L: 24 月龄草鱼精巢; M: 36 月龄草鱼精巢; N: 48 月龄草鱼精巢。

ED: 输精导管; Sv: 精小囊; Sg: 精原细胞; PS: 初级精母细胞; SS: 次级精母细胞; St: 精子细胞; Sp: 精子; T: 精巢。

A: Testis slice of 1-month-old grass carp; B: Testis slice of 2-month-old grass carp; C: Testis slice of 3-month-old grass carp; D: Testis slice of 4-month-old grass carp; E: Testis slice of 5-month-old grass carp; F: Testis slice of 6-month-old grass carp; G: Testis slice of 12-month-old grass carp; H: Testis slice of 24-month-old grass carp; I: Testis slice of 36-month-old grass carp; J: Testis slice of 48-month-old grass carp; K: Testis of 12-month-old grass carp; L: Testis of 24-month-old grass carp;

M: Testis of 36-month-old grass carp; N: Testis of 48-month-old grass carp.

ED: Efferent duct; Sv: Seminal vesicles; Sg: Spermatogonia; PS: Primary spermatocytes; SS: Secondary spermatocytes; St: Spermatoblast; Sp: Sperm; T: Testicle.

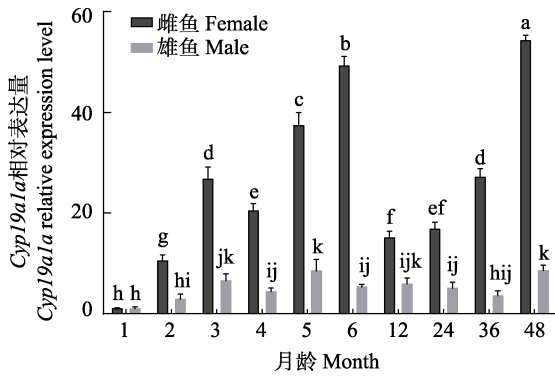


图3 不同时间段雌雄草鱼性腺 *cyp19a1a* mRNA 的相对表达变化

Fig.3 Expression variations of *cyp19a1a* mRNA in the gonads of male and female grass carp at different periods

不同字母表示各时间段基因表达差异显著($P < 0.05$)。下同。

Different letters indicate significant differences in gene expression at different period ($P < 0.05$). The same below.

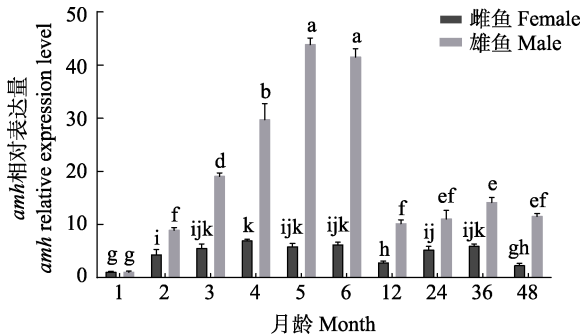


图4 不同时间段雌雄草鱼性腺 *amh* mRNA 的相对表达变化
Fig.4 Expression variations of *amh* mRNA in the gonads of male and female grass carp at different periods

3 讨论

3.1 草鱼原始性腺的发生和发育

与脊椎动物类似,大多数硬骨鱼类性腺最早的表现是生殖嵴的形成,随后原始生殖细胞通过迁移到达生殖嵴形成原始性腺,原始性腺进一步分化为卵巢和精巢(Piprek *et al.*, 2016)。不同鱼类的原始性腺形成时间存在差异,如花鲈(*Lateolabrax maculatus*)在55日龄时首次观察到原始性腺(李冰玉等, 2021);大黄鱼在20日龄时观察到原始性腺(游秀容等, 2012);军曹鱼(*Rachycentron canadum*)在15日龄时出现原始性腺的雏形(邝杰华等, 2021);鲫鱼(*Carassius auratus*) (岳敏娟等, 2009)的原始性腺则于16日龄形成。本研究中,1月龄草鱼在腹腔膜附近初次观察到生殖嵴。2月龄时在生殖嵴中可以观察到原始生殖细胞,标志着原始生殖细胞完成迁移,原始性腺正式形成,比同是鲤科的鲫鱼晚了大约44 d。结果表明,1~2月龄是草鱼原始生殖

细胞向生殖嵴迁移的关键时期。

原始性腺的形态变化也是研究鱼类性腺分化的重要特征之一,在大西洋鲑(*Salmo salar*)中,原始性腺横切面为圆形的普遍为雌鱼,横切面为三角形的普遍为雄鱼(赵维信等, 1994);革胡子鲶(*Clarias gariepinus*)原始性腺横切面呈梨形的普遍为雌性,横切面呈细条状的普遍为雄性(林光华等, 1995)。本研究中,生殖嵴形状为梨形且内部生殖细胞数量较多的为雌鱼,生殖嵴形状为椭圆状且生殖细胞数量较少的为雄鱼。这与军曹鱼(邝杰华等, 2021)、大菱鲂(*Scophthalmus maximus*) (Zhao *et al.*, 2017)、青鳞(*Oryzias latipes*) (Saito *et al.*, 2007)原始性腺的形态变化相似。

3.2 草鱼性腺发育的组织学研究

在大多数硬骨鱼类中,性腺分化时间是确定使用外源性激素性逆转成功的关键因素之一(Arslan *et al.*, 2009),因此,确定鱼类性腺分化时间可为鱼类激素诱导性逆转研究提供基础资料。硬骨鱼类性腺分化包括解剖学分化和细胞学分化的,解剖学分化的标志为性腺的形态结构发生变化,细胞学分化的标志为性腺中出现性母细胞(Strüssmann *et al.*, 2002)。一般情况下,鱼类性腺的解剖学分化早于细胞学分化(Li *et al.*, 2024),如大口黑鲈(*Micropterus salmoides*)解剖学分化开始于20日龄,细胞学分化开始于30日龄(Du *et al.*, 2022)。同种鱼类雌雄性腺分化时间也有差异,大部分鱼类卵巢分化早于精巢,如黄颡鱼卵巢分化最早出现于13日龄,而精巢分化则开始于55日龄(彭丽娜等, 2014)。也有部分鱼类精巢分化早于卵巢,如牙鲆(*Paralichthys olivaceus*)在50日龄精巢开始分化,65日龄时卵巢才开始分化(孙朝徽等, 2008)。黄河鲤(*Cyprinus carpio*)雌性性腺分化的起始时间为50~55日龄,雄性为90~100日龄(Jiang *et al.*, 2020)。本研究中,卵巢腔和输精导管等解剖学分化标志分别在3月龄和4月龄出现,比黄河鲤晚1个月左右。草鱼性腺中卵原细胞和精原细胞等细胞学分化标志分别在4月龄和5月龄出现,表明草鱼同大多数硬骨鱼类一样,性腺的解剖学分化早于细胞学分化,且雌性性别分化时间早于雄性。

鱼类完成性腺分化后,其性腺逐步发育至成熟。依据鱼类性腺的分期标准,可将草鱼卵巢和精巢分为6个时期。I期卵巢主要以卵原细胞为主,II期卵巢主要以初级卵母细胞和少量卵原细胞为主,III期卵巢以次级卵母细胞为主,IV期卵巢可观察到成熟的卵母细胞和卵黄颗粒,V期卵巢为产卵后的卵巢,VI期卵巢中可观察到退化后的卵细胞。I期精巢以输精管原

基和少量精原细胞为主, II 期精巢以精原细胞和少量初级精母细胞为主, III 期精巢以初级精母细胞和少量精子细胞为主, IV 期精巢以大量精子细胞为主, 小叶腔内可见少量成熟的精子, V 期精巢小叶腔内存在大量成熟的精子, VI 期精巢为产后的精巢(刘筠, 1993)。在本研究雌性草鱼的性腺组织切片中, 12 月龄观察到发育变大的卵原细胞; 24 月龄观察到初级卵母细胞; 36 月龄观察到次级卵母细胞, 卵黄颗粒清晰可见; 48 月龄观察到成熟的卵母细胞。在雄性草鱼的性腺组织切片中, 12 月龄时以精原细胞为主, 24 月龄时以初级精母细胞为主, 36 月龄可以观察到少量精子细胞; 48 月龄时精小囊破裂, 小叶腔中出现成熟精子, 由此可知, 12、24、36 和 48 月龄草鱼卵巢分别处于发育的第 I、II、III 和 IV 期, 精巢则分别处于发育的第 II、II、III 和 IV 期。至 48 月龄时, 华南地区雌雄草鱼性腺均已发育成熟。

3.3 性别特征基因 *cyp19a1a* 和 *amh* 的表达分析

cyp19a1a 基因是调控雌激素的关键因子之一, 在鱼类卵巢发育过程中发挥重要作用(Paixão *et al.*, 2022)。本研究结果显示, *cyp19a1a* 基因在草鱼卵巢中的表达总体呈先上升后下降再上升的趋势, 这与尼罗罗非鱼(*Oreochromis niloticus*)(Wen *et al.*, 2013)卵巢中的表达模式相似。2、3 月龄雌性草鱼性腺中 *cyp19a1a* 基因的表达量均呈显著升高趋势, 且该时期组织切片中结果显示草鱼雌性性腺由原始性腺逐渐分化形成卵巢腔, 表明 *cyp19a1a* 基因可能参与卵巢腔的形成过程。6 月龄时, 草鱼卵巢中卵原细胞大量增殖, 此时该基因表达量达到峰值, 表明 *cyp19a1a* 基因表达水平可能对卵原细胞的增殖具有促进作用。*cyp19a1a* 基因在 48 月龄时表达量最高, 此时草鱼卵巢已发育至 IV 期。该结果与黑鲷(*Acanthopagrus schlegelii*)(Liu *et al.*, 2004)中的研究结果相似, 推断 *cyp19a1a* 基因可能参与卵巢成熟过程。

amh 基因可促进生殖细胞增殖与分化, 在鱼类精巢分化过程中具有重要作用, 在精巢分化阶段持续高表达(韩玉龙, 2019)。本研究雄性草鱼性腺中 *amh* 基因表达水平从 2 月龄时开始持续上调, 5 月龄草鱼精巢中可观察到精原细胞, 此时 *amh* 基因表达水平达到峰值。这与 *amh* 基因在半滑舌鳎(*Cynoglossus semilaevis*)(刘姗姗等, 2013)和斑马鱼(*Danio rerio*)(Rodr *et al.*, 2005)早期性腺组织中的表达规律相似, 表明 *amh* 基因可能参与草鱼精原细胞的发生。也有研究表明, *amh* 基因表达于未成熟精巢的支持细胞中, 直至精巢成熟(Chen *et al.*, 2014)。本研究中, 草鱼 *amh* 基因表

达量在 6 月龄后显著下降, 并保持低表达至精巢成熟。大黄鱼(林爱强等, 2017)和大西洋鲑(Maugars *et al.*, 2008)的研究中也发现了类似现象, *amh* 基因在大西洋鲑未成熟的精巢中持续高表达, 至精子快速增殖时该基因表达量开始显著下降, 在精巢中精子量最多时表达量为最低。这可能是 *amh* 基因在抑制精子发生过程中也发挥了重要作用(Chen *et al.*, 2014)。

4 结论

本研究以不同月龄草鱼为研究对象, 通过组织切片技术观察不同时期草鱼性腺结构变化, 并结合荧光定量技术分析性别特征基因的表达, 明确了草鱼性腺发育启动时间约为 2 月龄, 雌雄性腺分化时间分别约为 3 月龄和 4 月龄, 至 6 月龄时雌雄草鱼性腺均已完全分化。12、24、36 和 48 月龄草鱼卵巢分别处于发育第 I、II、III 和 IV 期, 精巢则分别为发育第 II、II、III 和 IV 期。研究结果不仅丰富了草鱼的繁殖生理学资料, 也为激素诱导草鱼性别逆转技术研究提供了参考依据。

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Gonadal Histology and Expression Analysis of Sex Characteristic Genes in Grass Carp at Different Ages

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Abstract Grass carp belongs to Cypriniformes, Cyprinidae, and *Cyprinus* family, and is typically referred to as the four famous domestic fishes, black carp, silver carp, and bighead carp, respectively. The annual production of Chinese grass carp is consistently among the highest, with an output of 5.905 million tons in 2022, accounting for 24.3% of total of freshwater fish production. Reproduction is the core process of fish reproduction. Normal gonad development is a prerequisite for fish reproduction. At present, there are few reports on the sex differentiation of Chinese grass carp. Studying the differentiation and

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development of grass carp gonads can provide a typical model for gonadal development of cyprinid fish. In contrast, the start and completion time of gonadal differentiation are one of the key factors inducing sex reversal in fish using hormones. Research on gonadal differentiation and development in grass carp can also lay the foundation for the establishment of a unisexual population of grass carp.

Sex-related genes play important roles in biological processes such as sex determination, gonadal differentiation, and reproduction and development in fish. In most differentiated teleost fish, genes such as *dmrt1*, *sox9*, *foxl2*, *amh*, and *cyp19a1a*, are considered the key genes involved in sex regulation. *cyp19a1a* plays a key role in the gonadal differentiation and ovarian development of teleost fish by aromatizing androgens into estrogen via aromatase activity. This gene is specifically expressed in the gonads and is used served as a characteristic gene for sex differentiation towards females. Anti-Müllerian hormone (*amh*) regulates the structure of reproductive organs and the differentiation and development of reproductive cells. In fish such as carp, rainbow trout, and grass carp, the expression level of the *amh* gene in the testes is significantly higher than that in the ovaries, and is often used as a characteristic gene for male differentiation.

To explore the sex differentiation and developmental patterns of grass carp gonads, this study analyzed the tissue structure and expression differences of sex characteristic genes *cyp19a1a* and *amh* in the gonads of grass carp at 1, 2, 3, 4, 5, 6, 12, 24, 36, and 48 months of age using tissue sectioning technology and real-time fluorescence quantification technology. The results of tissue sectioning showed that grass carp exhibited reproductive ridges at 1-month-old. At 2 months of age, primitive germ cells were first observed in the reproductive crest, marking the formation of their primitive gonads. At 3-month-old, ovarian cavities and lobules were observed in the gonads of female grass carp. At 4-month-old, oogonia was observed, indicating anatomical differentiation at 3-month-old and cytological differentiation at 4-month-old. At the age of 4 and 5 months, ductus deferens and spermatogonia were respectively observed in male grass carp gonads, indicating anatomical differentiation at 4 months and cytological differentiation at 5 months. The ovaries of 24, 36, and 48-month-old grass carp were in the first, second, third, and fourth stages of development, respectively, while the testes were in the second, second, third, and fourth stages of development, respectively. At 48 months of age, the gonads of grass carp have matured. The fluorescence quantitative results showed that the expression level of the female characteristic gene *cyp19a1a* in the ovary showed an overall trend of first increasing, then decreasing, and then increasing again. The expression level of this gene was significantly upregulated at 2 months of age ($P < 0.05$), and reached its peak at 3, 6, and 48 months of age. During these three periods, anatomical changes were observed in the female gonads in the tissue sections, with a large proliferation of oocytes and the gonads tending to mature. The expression level of the male characteristic gene *amh* in the testes showed an overall trend of first increasing and then decreasing. At 2 months of age, the expression level of this gene was significantly upregulated ($P < 0.05$). At 5 months of age, it reached the peak when there were a large proliferation of spermatogonia in the gonads, and then remained low expression until gonadal maturity.

In summary, this study confirmed that the initiation time of grass carp gonadal development is approximately 2 months old, and the differentiation time of male and female gonads is approximately 3 and 4 months old, respectively. By the age of 6 months, both male and female grass carp gonads completely differentiated. These results not only enrich the reproductive physiology data of grass carp but also provide a reference basis for the study of hormone-induced sex reversal technology in grass carp.

Key words Grass carp; Sexual differentiation; Tissue slicing; Gene expression