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• 研究简报 •

## 江黄颡鱼卵细胞膜的结构特征

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### Structural characteristics of egg surface of *Pelteobagrus vachelli*

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**Abstract:** Using conventional histochemical methods and scanning electron microscope, the egg surface of mature oocyte of *Pelteobagrus vachelli* was observed. The micropyle is funnel-like and consists of micropylar vestibule and micropylar canal. The opening of micropylar vestibule is about 11.35  $\mu\text{m}$  in diameter while entrance of the micropyle to the oocyte is only about 2.6  $\mu\text{m}$ . There are plenty of micropores on the surface near the micropyle. Micropyle might act as an apparatus leading spermatozoa to penetrating an oocyte. Regular cord-like structures between basal membrane (the outermost layer of egg surface) and plasmic membrane (the inner layer of egg surface) are found. These cord-like structures, each about 16.75 to 18.50  $\mu\text{m}$  in length, 5  $\mu\text{m}$  in diameter, comprised small balls whose diameters vary from 2.18 to 2.5  $\mu\text{m}$  and are arranged compactly under the basal membrane. These kinds of structures are zona radiata and act as special eggshell. After activation of the oocytes, the cord-like structures would partially transform into mucoprotein. To our best knowledge, this paper is the first report on stereo view of the zona radiata of teleost oocyte.

**Key words:** *Pelteobagrus vachelli*; zona radiata; micropyle; egg surface; ultrastructure

目前对硬骨鱼类卵子的表面结构已有不少报道<sup>[1-6]</sup>, 并已初步应用于系统分类学<sup>[7-10]</sup>和环境监测<sup>[11-13]</sup>等领域, 卵细胞膜表面的超微结构和卵孔特征也被用作鉴定鱼卵和鱼类产卵类型的依据<sup>[10,14]</sup>。但由于不同鱼类的卵表各具特点, 而且对卵子表面结构的观察受到研究方法和手段的制约, 至今在鱼类卵细胞表面的具体结构的细微特征等方面仍存在不少疑问, 如鱼类卵膜辐射带的立体结构尚未见报道。本文对江黄颡鱼(*Pelteobagrus vachelli*) (鲇形目、鲇科、黄颡鱼属)的卵细胞表面进行了超显微结构研

究, 初步描述了江黄颡鱼的卵表结构, 发现了其卵膜辐射带的特殊结构特征, 并对其功能进行了初步分析。

### 1 材料与方法

实验于2001年4-6月进行。实验所用的江黄颡鱼取自安徽省淮南市窑河渔场, 为人工饲养2~3年的成鱼, 体长30~50 cm不等。解剖雌鱼, 取出成熟的卵巢并快速用PBS漂洗后, 在冰袋上切成小块, 迅速分散成单个或小团卵细胞, 经PBS配置的3%戊二醛初固定后, 置4℃避

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光保存。在扫描电镜观察前用 PBS 漂洗 3 次,1% 钨酸后固定 1 h,酒精梯度脱水,临界点干燥仪干燥,离子溅射仪镀膜,应用 JEOL JSM-T300 型扫描电子显微镜观察卵细胞表面结构,加速电压为 15 kV。

取江黄颡鱼的成熟卵巢,用上述方法固定和处理。在酒精梯度脱水时快速脱水,每个酒精梯度的脱水时间不长于 5 min。此时有部分受精卵表面破裂。用上述方法观察卵膜断面的结构。取江黄颡鱼人工授精后 30 s 的受精卵,用上述方法观察卵膜结构。

另取部分性腺样本用 Bouin 氏液固定,石蜡包埋,连续切片,H. E 染色,Olympus 显微镜观察并拍照。

## 2 结果

### 2.1 卵孔

江黄颡鱼的成熟卵细胞具有卵孔(micropyle)(图版-1),卵孔由卵膜内陷而成,包括卵孔管和卵孔前庭(图版-1 a, b)。卵孔呈漏斗状,此处辐射带变薄甚至消失,卵细胞核与之靠近(图版-2),这有利于受精时精原核与卵原核的结合。卵孔外部孔径约 11.35  $\mu\text{m}$ ,向内逐渐变小,入口处仅约 2.60  $\mu\text{m}$ (图版-1, 2)。受精后 30 s 出现塞状结构(plug-like structure),将卵孔封闭(图版-3 a)。

### 2.2 卵表结构

在卵细胞的卵膜上,存在大量的微孔,直径约 0.3  $\mu\text{m}$ (图版-3 b)。微孔是卵细胞在卵巢中与体内环境进行物质和信息交换的通道。

### 2.3 卵膜层的一种特殊结构

在成熟卵细胞膜表面裂口观察到一种特殊的有规则结构。这种特殊结构的基本单位为小球状,每个小球直径约 2.18~2.50  $\mu\text{m}$ ;约 12~13 个小球纵向致密排列,横向由 4~6 个小球错落有致的组成,由此构成一索状单元(图版-4),索状单元厚度约 16.75~18.50  $\mu\text{m}$ 。许多索状单元紧密相连,排列在基膜下(图版-5)。

## 3 讨论

### 3.1 卵膜层的特殊构造可能是辐射带

根据索状结构的厚度及所在位置等推测,实验所见的特殊结构应为辐射带的局部结构。推测依据如下:

(1) 特殊结构所在位置与辐射带(图版-6)所在位置一致。卵细胞在卵巢内没有游离时,外层有双层细胞,最外一层的细胞为扁平状(图版-7 a),第二层为滤泡细胞,呈球状(图版-7 b);再往里有一基膜(图版-7 c),为非细胞结构,然后才是辐射带(图版-7 d)。江黄颡鱼的卵细胞排出体外以后,滤泡膜不随同卵子排出(图版-8, 9)。因此,排出体外的成熟卵细胞膜最外层为基膜,向里为辐射带、质膜等。索状结构(图版-4 b)的外层正是非细胞结构的基膜(图版-4 a),而出现在索状结构下方的为质膜(图版-4 c)。特殊结构正好处于两者中间,因此与辐射带位于同一位

置。

(2) 特殊结构的厚度与辐射带切面厚度一致,经测量两者厚度约为 17.5  $\mu\text{m}$ 。

(3) 辐射带的纵切面可见纵纹(图版-6),其宽度约 3.5  $\mu\text{m}$ ,这与索状结构的直径正好一致。

根据以上几点推测,可以认为本实验所见的索状结构所构成的膜就是辐射带。

### 3.2 辐射带的生化组成和功能

硬骨鱼类卵母细胞的卵黄膜(vitelline envelope)由表皮细胞、滤泡细胞、基膜、辐射带、质膜组成<sup>[15]</sup>。辐射带是一种复杂的细胞外基质,主要由多种蛋白及许多特异的大分子糖蛋白、硫酸化和羧基化的酸性粘多糖组成<sup>[4,16]</sup>。辐射带具有微孔管(micropore canal),微孔管内充满卵细胞的微绒毛(microvilli)和滤泡细胞的突起,这是鱼类对卵细胞的发育进行调控以及卵细胞与内环境进行物质和信息交换的通道<sup>[16]</sup>。辐射带的蛋白由雌鱼的肝脏合成,受雌二醇调控,功能与哺乳动物的透明带相似,且鱼类的辐射带蛋白的编码基因与哺乳动物卵细胞透明带的编码基因有很高的同源性<sup>[17]</sup>。

硬骨鱼类辐射带的厚度由几微米到数十微米不等,在胎生的鱼类,辐射带和卵膜很薄,这有利于胚胎与母体之间进行物质交换;而卵生鱼类的辐射带相对较厚,厚的辐射带对受精卵起明显的保护作用<sup>[15]</sup>。由于卵母细胞是一种体积很大的细胞,达到生理成熟后,脱离滤泡膜的保护,游离在卵巢腔中,此时卵细胞必须承担相当大的体压;当卵细胞通过生殖孔排出体外后,又必须面对渗透压的调节和卵子形状的维持等问题,因此每个卵细胞都必须具备某种完善的生物力学结构的物质基础。江黄颡鱼的卵细胞直径约为 1.8 mm,是一般动物细胞体积的数十万倍,而且要经历特殊的生理过程,很难想象一般的细胞骨架系统能满足卵细胞稳定的需要。因此作者认为,在排卵后,由于卵细胞与滤泡膜分离,此时起保护和稳定作用的主要结构基础是辐射带,辐射带具有类似蛋壳的作用。从辐射带的结构和生化组成看来,辐射带无论从几何构型,还是从化学组成,都能起到稳定的功用。

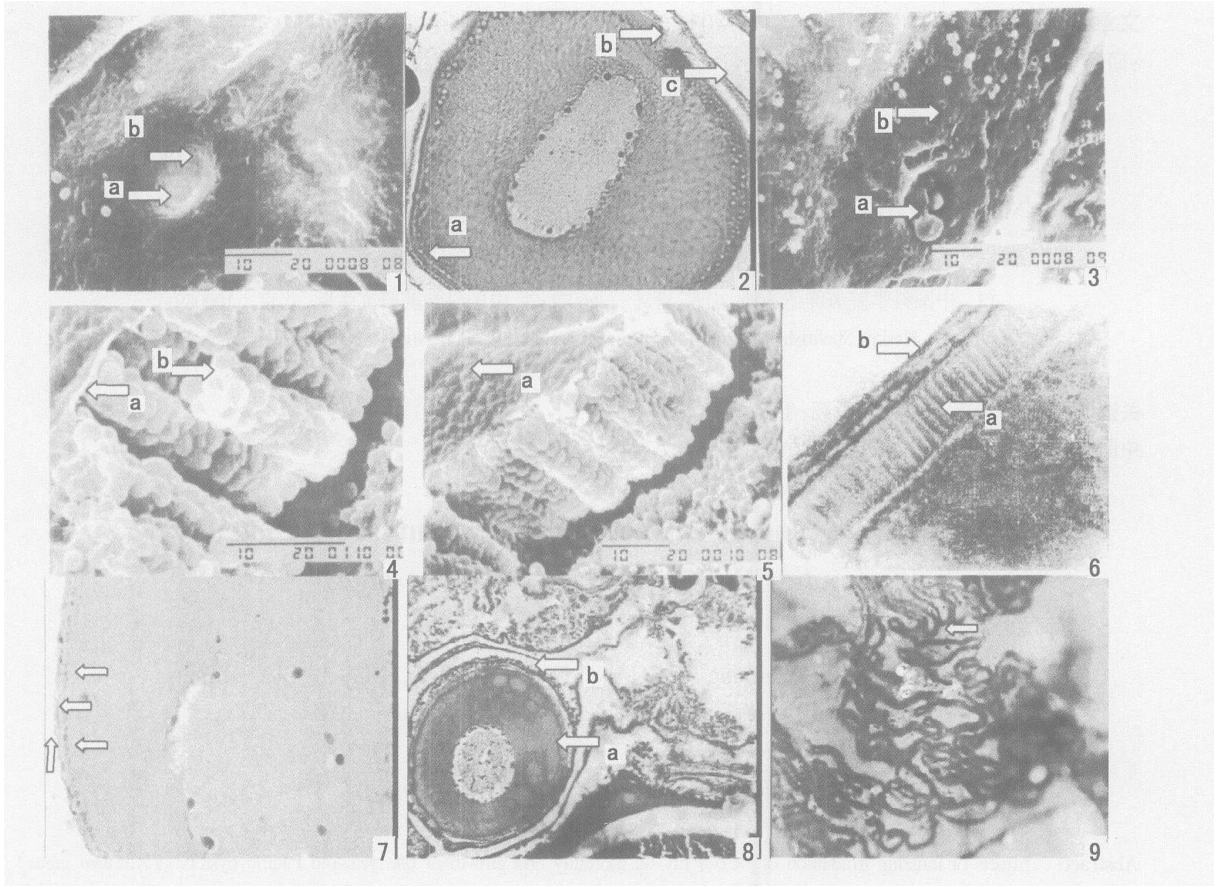
此外,当卵子被激活后,卵表发生皮质反应而释放出酸性粘多糖和多种蛋白,形成受精膜,这层膜具有较强的抗菌活性,它也起源于辐射带<sup>[1,18]</sup>,而且,辐射带还与鱼卵的粘性相关<sup>[8]</sup>。

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### 图版说明 Explanation of Plate

1. 卵孔: a, 卵孔管; b, 卵孔前庭。Bar = 10 μm; 2. 卵孔纵视图: a, 辐射带; b, 卵孔; c, 滤泡膜。× 200; 3. 受精后的卵孔及卵膜: a, 塞状结构; b, 卵膜表面的微孔。Bar = 10 μm; 4. 卵膜的特异结构: a, 基膜; b, 索状结构; c, 质膜。Bar = 10 μm; 5. 索状单位构成辐射带: a, 基膜。Bar = 10 μm; 6. 辐射带剖面结构: a, 辐射带; b, 滤泡膜。× 1000; 7. 卵膜的结构: a, 上皮细胞; b, 滤泡细胞; c, 基膜; d, 辐射带; 8. 产卵后的卵巢, 卵细胞与滤泡膜分离: a, 卵细胞; b, 滤泡膜; 9. 产卵后滤泡膜留在体内

1. plan view of the micropyle. a, micropylar canal; b, micropylar vestibule. Bar = 10 μm; 2. longitudinal view of the micropyle. a, zona radiata; b, micropyle; c, follicular epithelium. × 200; 3. micropyle and egg envelope post fertilization. a, plug-like structure in the micropyle; b, micropores on the surface of egg envelope. Bar = 10 μm; 4. showing the strange conformation of the envelope. a, basal membrane; b, cord-like unit of the envelope; c, plasma membrane Bar = 10 μm; 5. cord-like structures consisting of zona radiata. a, basal membrane. Bar = 10 μm; 6. longitudinal view of zona radiata. a, zona radiata; b, follicular epithelium. × 1000; 7. the egg envelope. a, epithelial cell; b, follicular cell; c, basal membrane; d, zona radiata; 8. an oocyte being left in the ovary after spawning, showing the oocyte departing from the follicular epithelium. a, oocyte; b, follicular epithelium; 9. the follicular epithelium was left in the ovary after spawning