第11卷第4期
2004年7月

中国水产科学
Journal of Fishery Sciences of China

Vol. 11 No. 4
July 2004

施氏鲟不同年龄性腺发育与性类固醇激素浓度关系

孙大江，曲秋芝，王丙乾，马国军
（中国水产科学研究院 黑龙江水产研究所，黑龙江 哈尔滨，150070）

摘要：采用放射免疫方法测得养殖施氏鲟群体5个年龄组及亲鱼（8龄）血液中睾酮（Testosterone，T）、雌二醇（17β－estradiol，E_2）的不同含量，并与其对应年龄的性腺组织学观察进行分析。结果显示，1龄性腺刚刚完成性分化发育初期，T（$\bar{X}\pm SD$）=（1.8±0.724）nmol/L，$\alpha=0.01$；E_2（$\bar{X}\pm SD$）=（55.8±2.879）pmol/L，$\alpha=0.05$。2龄精巢Ⅱ期，卵巢Ⅰ期，T（$\bar{X}\pm SD$）=（2.2±0.934）nmol/L，$\alpha=0.01$。E_2（$\bar{X}\pm SD$）=（38.9±2.343）pmol/L，$\alpha=0.05$。3～4龄鱼卵巢发育在Ⅰ～Ⅱ期，精巢Ⅲ－Ⅳ期。3龄T（$\bar{X}\pm SD$）=（9.6±1.936）nmol/L，$\alpha=0.05$；E_2（$\bar{X}\pm SD$）=（44.8±2.605）pmol/L，$\alpha=0.05$。4龄鱼T（$\bar{X}\pm SD$）=（26.3±2.105）nmol/L，$\alpha=0.05$；E_2（$\bar{X}\pm SD$）=（55.3±3.053）pmol/L，$\alpha=0.05$。5龄鱼卵巢发育在Ⅲ期左右，精巢已发育成熟，T（$\bar{X}\pm SD$）=（13.9±1.652）nmol/L，$\alpha=0.05$。E_2（$\bar{X}\pm SD$）=（137.7±5.880）pmol/L，$\alpha=0.05$。雄亲鱼血液中T含量变化在40.5～74.6 nmol/L，E_2含量变化在56～116 pmol/L。雌亲鱼血液中T含量变化为39.7～73.2 nmol/L，E_2含量变化在544～904 pmol/L。

关键词：施氏鲟；性腺；睾酮；雌二醇
中图分类号：Q959.463　　**文献标识码**：A　　**文章编号**：1005－8737－（2004）04－0307－06

施氏鲟（*Acipenser schrenckii*）隶属鲟形目（Acipenseriformes）、鲟科 Acipenseridae、鲟属 *Acipenser*，分布于黑龙江，是大型的淡水鱼类之一，为江河定居种类。近年来随着养殖技术的不断完善，施氏鲟已经成为养殖新品种，养殖产量逐渐提高。同时开展了一些相关的研究工作，有人工繁殖、染色体核型及消化道组织结构等[1-4]。但有关性腺发育及性类固醇激素分泌的研究未见报道。本研究对施氏鲟性腺发育进行组织学观察，同时监测了发育不同阶段血液中性类固醇激素含量的变化，对两者之间的动态关系进行论述。

1　材料与方法

1.1　材料

2002年5月，采集1～5龄及成熟亲鱼，饲养在中国水产科学院鲟鱼繁育技术工程中心的室外水泥池中，平均水温：12月6 ℃，1月4 ℃，7月24 ℃，8月28 ℃。长日照13.5 h（7月），短日照9 h（12月），商品饲料饲养。1～5龄鱼10～15尾，亲鱼雄3尾，雌3尾。每尾鱼分别采血2 mL，然后取性腺组织（包括精巢和卵巢）观察外部形态。

1.1.1　样品制备　性腺用Bouin氏液固定，梯度酒精脱水，二甲苯透明，石腊包埋。同时用松油醇透明，石腊包埋。切片4～8 μm。火棉胶涂膜，苏木素伊红染色。显微镜下观察、测量（包括计数）。

1.1.2　测定方法　卵巢发育的分期依据，采用前苏联学者 Детлаф[5] 和孙大江等[6] 提出的分期标准，把卵巢发育分为Ⅰ～Ⅵ期。卵巢发育各期的界定，是以在卵巢切面中所占面积超过50%的生殖细胞的时相为准。精巢发育期参照前苏联学者 Детлаф[5] 提出的雄鱼成熟度分期标准。

1.1.3　放射免疫测定方法　取各血清样品40 μL，分别加入2个测定管中，每管20 μL。各测定管加入相应的氚标记类固醇，100 μL相应的抗血清，4 ℃过夜保存，再加入0.5 mL活性炭葡聚糖。40 min旋转混合后，离心（3 000 r/min，10 min），各管吸出上清液放入闪烁杯内，各加10 mL闪烁液，用LKB1217RACKBETA LIQUID ' SCIEVTILLATION COUNTER液闪计数仪计数。

运用 Simpon 和 Wright[7-8] 建立的放射免疫（RIA）测定法进行血清中雌二醇（17β－estradiol，E_2）和睾酮（Testosterone，T）浓度的测定。氚标记类

收稿日期：2003－10－10；　**修改日期**：2004－02－27.
基金项目：科技部"十五"科技攻关计划（2001BA5050506）.
作者简介：孙大江（1955－），男，研究员，从事水产养殖生物学研究，Tel：0451－84607274，E－mail：sundajiang0451@sohu.com

固醇激素试剂盒由世界卫生组织提供。

2 结果

2.1 性腺分化早期组织学

在性分化早期(1龄),性腺很小,呈透明的细线状,肉眼无法分辨精巢或卵巢。光学显微镜下切片观察,可见精巢和卵巢呈不同的形态结构,性腺生殖上皮向内凹陷,纵向与性腺长轴平行形成沟槽,切面呈深度凹陷或锯齿状结构,生殖上皮由1层或2层柱状细胞构成,柱状细胞下是进行有丝分裂的卵原细胞增殖团,此结构为卵巢原基[5,9](图版Ⅰ-1)。相反没有这种结构且性腺生殖上皮表面光滑饱满,生殖细胞群位于性腺的内部,这种形态结构的性腺是精巢[9-11]。精巢内出现原始精小叶,小叶内的生殖细胞处于初级精原细胞阶段,精巢发育为Ⅰ期(图版Ⅰ-2)。

2.2 精巢组织学观察

2.2.1 Ⅱ期精巢(2龄) 精巢呈白色半透明细带状,与脂肪组织紧密相联,肉眼很难鉴别脂肪与性腺。切片观察精巢内初步形成精小叶,精小叶内充满初级和次级精原细胞,次级精原细胞增多,体积略小。在精小叶中成群排列,精小叶均匀分布在精巢内(图版Ⅰ-3)。

2.2.2 Ⅲ期精巢(3龄) 精巢已发育成腺体形,表面富有弹性并分布许多血管,淡粉色。切片观察精小叶出现空腔。初级精母细胞沿小叶边缘呈单层或多层排列,细胞直径与次级精原细胞无明显差别(图版Ⅰ-4)。

2.2.3 Ⅳ期精巢(4龄) 性腺很发达,约占腹腔的1/2,乳白色。切片观察可以见到由次级精母细胞、精细胞和精子组成的精小囊。小囊内的细胞群处于相同发育期。小囊的细胞数量明显增多(图版Ⅰ-5)。

2.2.4 Ⅴ期精巢(5龄) 精巢呈膨胀的软体状,乳白色,鱼体离水时,轻轻挤压便能从泄殖孔流出乳白色精液。切片显示各精小叶扩大,充满成熟的精子(图版Ⅰ-6)。

2.3 卵巢组织学观察

2.3.1 第Ⅰ期卵巢(2~3龄) 卵巢呈白色半透明窄带状与脂肪组织相近,肉眼仍不能分辨精巢或卵巢。显微镜下切片观察,卵巢中的生殖细胞是以第一时相的卵原细胞为主,细胞质很少,细胞直径在10~60 μm,最小的卵原细胞直径10 μm,细胞核直径7.5 μm,细胞核大占据了卵原细胞的大部分体积,核直径为7.5~38.5 μm,核内染色质呈丝状均匀分布,又可称染色质核期。随着卵原细胞的发育,细胞质渐渐增多,并表现了明显的嗜碱性和苏木精染色易着色性。卵原细胞分布在卵巢表层组织结构中(版图Ⅰ-5,6)。

2.3.2 第Ⅱ期卵巢(4~5龄) 卵巢乳白色中略见淡淡黄色,肉眼可见半透明状针眼大的卵粒,低倍光镜下能见到丰富的脂肪组织包裹着卵巢,卵巢表面分布着大小不同的卵。切片观察,卵巢组织内也有大量的脂肪,占卵巢整体的大部分。卵巢中的生殖细胞是以第二时相的初级卵母细胞小生长期的细胞为主,细胞呈多角圆形,初级卵母细胞直径为60~200 μm,细胞质增多并嗜碱性,核也相应增大,核直径为38.5~82.5 μm,核仁(7~26个)靠近核膜内侧环形分布,也可称为外周核仁期[8]。部分细胞质可见到网状结构。初级卵母细胞外面已有滤泡膜。随着初级卵母细胞的生长发育,细胞质的嗜碱性减弱,少部分初级卵母细胞很大,卵径在400 μm左右(图版Ⅰ-7,8)。

2.4 睾酮、雌二醇含量

施氏鲟血液中T与E_2含量测量结果如图1所示,T在血液中的含量随着年龄增长而增加以($\bar{X}\pm$SD)表示,1龄为(1.764±0.724) nmol/L,$\alpha=0.01$;2龄(2.180±0.934) nmol/L,$\alpha=0.01$;3龄(9.6±1.936) nmol/L,$\alpha=0.05$;4龄(26.3±2.105) nmol/L,$\alpha=0.05$;5龄(13.9±1.652) nmol/L,$\alpha=0.05$。从1龄到4龄T含量是处于上升状态,到5龄时表现了下降趋势。E_2与T含量有所不同,以($\bar{X}\pm$SD)表示,1龄(55.8±2.879) pmol/L,$\alpha=0.05$;2龄(39.1±2.343) pmol/L,$\alpha=0.05$;3龄(44.8±2.605) pmol/L,$\alpha=0.05$;4龄(55.3±3.053) pmol/L,$\alpha=0.05$;5龄(137±5.870) pmol/L,$\alpha=0.05$;E_2 1龄时维持较低水平,到2龄时下降到更低的水平,2~4龄E_2水平缓慢上升,4~5龄E_2水平快速上升。8龄亲鱼生产时雌雄个体的T含量均达到较高水平(表1)。

雌性亲鱼可达到904 pmol/L,雄性亲鱼的17β-E_2维持在较低的水平内(见表1)。

3 讨论

3.1 性腺发育

与大多数硬骨鱼类相同,鲟科鱼类性分化后雄

性个体性腺发育较雌性的性腺发育快[12-14]。施氏鲟性腺组织学观察结果:精巢发育,1 龄性分化初期,精原细胞发生;2~4 龄精巢从Ⅱ期发育到Ⅳ期;5 龄达到性成熟,精子形成。卵巢发育,2~3 龄Ⅰ期卵巢以卵原细胞为主;4~5 龄Ⅱ期卵巢以初级卵母细胞小生长期的细胞为主,8 龄性腺成熟[1]。而自然群体雄性最小成熟年龄 7~8 龄[6],雌性最小成熟年龄 9 龄[15]。人工养殖条件下,施氏鲟生殖群体性腺成熟提早 1 年。Stearn[16]指出,鱼类性成熟的年龄和成熟时躯体的大小并非固定,而是具有一定的可塑性。生长状况良好的雌雄个体均有可能提前 1~2 年性成熟。在同一种群中鱼类性成熟年龄的变异有可能来自遗传的差异[17],也可能来自环境的影响[18]。Wootton[19]指出,当环境的变化使生长率增高时,所产生的效应通常是使性成熟年龄提前。

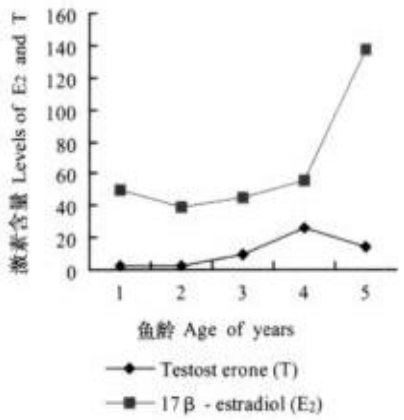


图 1　各龄鱼血液中性激素含量变化

Fig. 1　Changes in serum levels of E_2 and T from 1 to 5 years of age

表 1　亲鱼(8 龄)血液中睾酮与雌二醇含量

Table 1　E_2 and T concentration in serum of stocks

项目 Item	♂1	♂2	♂3	$\bar{x}$	♀1	♀2	♀3	$\bar{x}$
睾酮/(nmol·L^{-1}) Testosteron	74.6	47.6	40.5	54.2	56.2	39.7	73.2	56.4
雌二醇 17β-estradiol/(pmol·L^{-1})	116	59	56	77	544	774	904	777.3

3.2　睾酮、雌二醇含量与性腺发育的关系

性类固醇激素对鱼类生殖过程的各个时期都有影响。促性腺激素对生殖细胞发育成熟的调节主要是间接通过类固醇激素的作用。性类固醇激素的分泌量,直接反应性腺发育状况[20]。本研究表明性腺发育早期 1 龄时刚刚完成性分化,血液中的 T 含量处于较低的水平;2~3 龄精巢发育Ⅱ~Ⅲ期,卵巢发育Ⅰ期,T 含量开始上升;4 龄时精巢发育Ⅳ期,卵巢发育Ⅱ期 T 含量达到峰值;5 龄精巢发育成熟,卵巢仍处于Ⅱ期,T 含量下降。1 龄 E_2 含量较低;2 龄 E_2 处于最低水平;3 龄 E_2 含量缓慢上升;4 龄 E_2 含量较 1 龄含量略高;5 龄 E_2 含量呈直线上升。8 龄亲鱼雄性个体 T 含量均处于较高的水平,E_2 含量相应较低;雌性个体 T 含量同样很高,E_2 含量最高。林浩然[21]认为睾酮是雌激素前体。Crim 等[22]曾认为在未成熟的虹鳟,雄激素能促使脑垂体的 GtH 合成是芳化酶的作用,将睾酮芳化为雌二醇。Fostier 等[23]认为雌或雄亲鱼血液中 T 含量均很高,是因为硬骨鱼类能够合成 T,并将 T 转变成 E_2。

参考文献:

[1] 曲秋芝,孙大江,马国军,等. 施氏鲟全人工繁殖研究初报[J]. 中国水产科学,2002,9(3):277-279.

[2] 宋苏祥,刘洪柏,孙大江,等. 施氏鲟的核型及 DNA 含量研究[J]. 遗传,1997,19(3):5-8.

[3] 曲秋芝,华育平,曾朝辉,等. 史氏鲟消化系统形态学与组织学观察[J]. 水产学报,2003,27(1):1-4.

[4] 叶继丹,刘洪柏,赵吉伟,等. 史氏鲟及杂交鲟仔鱼消化系统的组织学[J]. 水产学报,2003,27(2):177-182.

[5] Детлаф Т А и, Гинзбург А С.(张贵寅,赵尔宓译). 鲟鱼类的胚胎发育与其养殖问题[M]. 北京:科学出版社, 1958:26-46.

[6] 孙大江,曲秋芝,吴文化,等. 史氏鲟人工繁殖及养殖技术[M]. 北京:海洋出版社, 2000. 12-16.

[7] Simpson T H, Wright R S. A radioimmunoassay for 11-oxo-estosterone: its application in the measurement of levels in bood serum of rainbow trout *S. gairdneri*[J]. Steroids, 1977, 29:383-398.

[8] Wdright R S, Hunt S M V. A radioimmunoassay for 17α20β-dihydroxy-4-pregnen-3-one: its use in measuring changes in serum level at ovulation in Atlantic salmo *Salmo salar*, Coho salmon *Oncorhynchu skisutch*, and rainbow trout *Salmo gairdneri*[J]. Gen Comp Endocrinol, 1982, 47:475-482.

[9] Naotaka O, Mamoru M, Koji Y, et al. Histological observations of gonadal sex differentiation in the F_2 hybrid sturgeon, the bester[J]. Fisheries Science, 2001;67:1 104-1 110.

[10] Naotaka O, Mamoru M, Koji Y, et al. Effects of estradiol-17β and 17α-methyltestosterone on gonadal sex differentiation in the F_2 hybrid sturgeon, the bester[J]. Fisheries Science, 2002, 68:

1 047 – 1 054.
[11] Mojazi Amiri B, Maebayashi M, Adachi S, et al. Testicular development and serum sex steroid profiles during the annual sexual cycle of the male sturgeon hybrid[J]. J Fish Biol, 1996, 48: 1 039 – 1 050.
[12] Akimova N B, Malyutin V S, Smolyanov I I, et al. Growth and gametogenesis of the Siberian sturgeon *Acipenser baeri* under experimental and natural condition[J]. Aquaculture, 1978, 9: 179 – 183.
[13] Kijima T, Maruyama T. Histological research for the development of the gonad of hybrid sturgeon, Bester (*Acipenser ruthenus* L. male × *Huso huso* L. female)[J]. Bulletin of National Research Institute of Aquaculture 1985: 8,23 – 29.
[14] Kijima T, Fujii K, Maruyama T, et al. Histological studies of the gonad of 1 – ,2 – and 3 – year – old hybrid sturgeon, Bester between female *Huso huso* and male *Acipenser ruthenus*[J]. Bulletin of National Research Institute of Aquaculture. 1988, 14, 133 – 138.
[15] 任慕连. 黑龙江鱼类[M]. 哈尔滨: 黑龙江人民出版社, 1981. 5 – 10.
[16] Steam S C, Cradall R E. Plasticity for age and size at sexual maturity: a life – history response to unavoidable stress[A]. Fish Reproduction: Strategies and Tactics[C]. London: Academic Press, 1984,99:13 – 33.
[17] Alm G. Connection between maturity size and age in fishes[J]. Rep Inst Freshwat Res Dorttningholm, 1959,40:5 – 45.
[18] Pitt T K, Changes in abundance and certain biological characters of Grand Bank American plaice, *Hippoglossoides plaslessoides*[J]. Fish Res Bd Can, 1975,32:1 383 – 1 389.
[19] Wootton R J, Reproduction in Ecology of Teleost Fishes[M]. London: London Chapman and Hall Ltd, 1993,99:159 – 195.
[20] Amiri B M, Maebayashi M, Adachi S, et al. In vitro steroidogenesis by testicular fragments and ovarian follicles in a hybrid sturgeon, Bester[J]. Fish Physiology and Biochemistry, 1999, 21:1 – 14.
[21] 林浩然. 鱼类生理学[M]. 广州: 广东省高等教育出版社, 1999,190 – 193.
[22] Crim L W, Peter R E, Billard R. Onset of gonadotropic hormone accumulation in the immature trout pituitary in response to estrogen or aromatizabde androgen steroid hormones[J]. Gen Comp Endocrinol, 1981,44:374 – 381.
[23] Fostier A, Jalabert B, Billard R, et al. The gonadal steroids[A]. Fish Physiology[C]. New York: Academic Press, Vol. Ⅸ A 1983. 277 – 372.

Relationships between gonad development and sex steroids level at different age of *Acipenser schrenckii*

SUN Da-jiang, QU Qiu-zhi, WANG Bing-qian, MA Guo-jun
(Heilonjiang River Fishery Research Institute, Chinese Academy of Fishery Sciences, Harbin 150070, China)

Abstract: Amur sturgeon *Acipenser schrenckii* was distributed in Heilongjiang River. With the gradual perfection of culture technique, *Acipenser schrenckii* had been a new species in terms of aquaculture. The culture yields have increased gradually. Some scientists have worked on some relative studies such as artificial propagation, embryonic and larval development and histological observations of digestive system. But no substantial information is available on gonad and related sex steroids for Amur sturgeon. The gonad development at all stages were influenced by sex steroid hormone. Study on sex steroid level in serum can provide new information on the testicular and ovarian steroidogenic capacity of sturgeon, and may serve as the basis for further studies on the hormonal control of male and female gonadal development in this fish. The present study deals with the results of histological observations on the gonad development and the serum sex steroids levels from 1 to 5 years of age and the stocks were also examined, to obtain preliminary information on endocrine control of gonad development and elucidate the most suitable time for induction of artificial propagation. In May 2002, the fish used in this study at 1 to 5 years of age were stocked and raised in outdoor cement pool in the Technological and Engineering Center of Sturgeon's Reproduction , Beijing, China, under natural water temperature and day length. The lowest monthly average water temperature was 6 ℃ in December and 4 ℃ in February, and the highest was 24 ℃ in June and 28 ℃ in August. The longest day time was 13.5 h (June) and the shortest was 9 h (December). The fish were fed commercial sturgeon food. The fragments of testes and ovarian, both from 1-to 5-year-old fish, were collected randomly by biopsy. Portions of gonad, from approximately the same area in the middle part of the organs, were removed through a surgical incision 5 – 8 cm

long laterally on the abdomen. The blood was collected from the caudal vein of each fish. The gonad development stage was determined by light microscopy according to Детлаф and SUN. The gonad fragments were fixed in Bouin's solution for 48 h, and embedded in cytoparaffin after dehydration and clearing. Serial sections of 4 – 7 μm thickness were stained with Delafield's haematoxylin and eosin. The levels of testosterone (T) and estradiol – 17β (E_2) were measured by radioimmunoassays (RIA) according to Simpon and Wright. The results showed that: at age 1, which was the early stage of sex differentiation, the levels of testosterone (T) and 17β – estradiol (E_2) were (1.8 ±0.724) nmol/L(α =0.01) and (50.3 ±2.879) pmol/L(α =0.05), respectively. At age 2, the testes were at stage Ⅱ and the ovarian was at stage Ⅰ when the levels of T and E_2 were (2.2 ±0.934) nmol/L (α = 0.01) and (38.9 ±2.343)pmol/L(α =0.05), respectively. At age 3, the testes were at stage Ⅲ and the ovarian was at stage Ⅰ, and the levels of T and E_2 were (9.6 ±1.936) nmol/L (α =0.05) and (44.8 ±2.605) pmol/L (α =0.05), respectively. At age 4, the testes were at stage Ⅲ and the ovarian at stage Ⅱ when T and E_2 levels were (26.3 ±2.105) nmol/L (α =0.05) and (55.3 ±3.053) pmol/L (α =0.05), respectively. At age 5, the testes were mature and the ovarian were at stage Ⅲ when the levels of T and E_2 were (13.9 ±1.652) nmol/L(α = 0.05) and (137.7 ±5.880) pmol/L (α =0.05), respectively. The levels of T and E_2 were 40.5 – 74.6 nmol/L and 56 – 116 pmol/L, respectively, in the male stocks, and 39.7 – 73.2 nmol/L and 544 – 904 pmol/L in the female stocks, respectively.

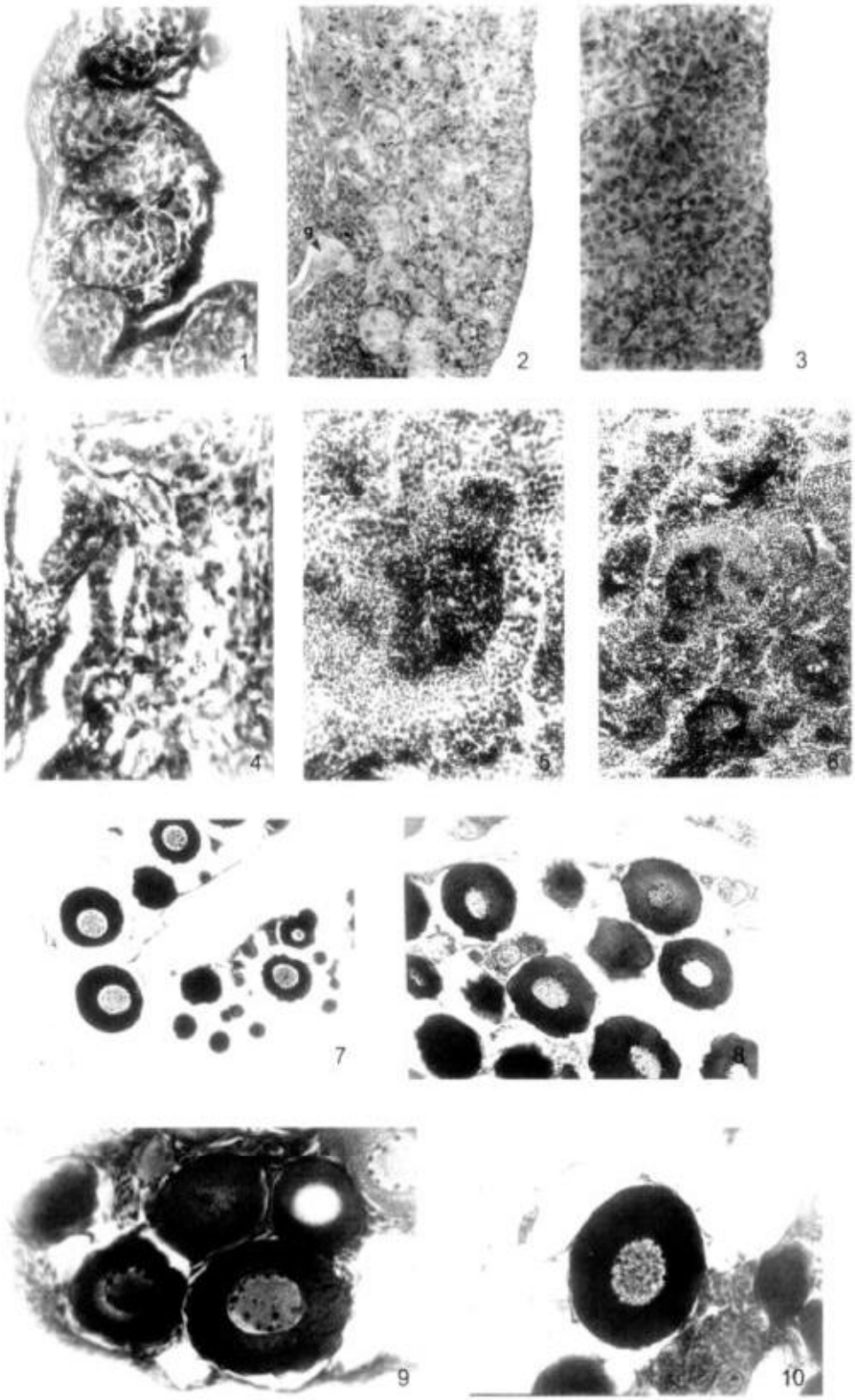
Key words: *Acipenser schrenckii*; sex gonad; testosterone ; 17β – estradiol

图版Ⅰ说明

1. 性分化早期卵巢,生殖细胞增殖团, ×400;
2. Ⅰ期精巢, ×400;
3. Ⅱ期精巢, ×400;
4. Ⅲ期精巢, ×400;
5. Ⅳ期精巢, ×400;
6. Ⅴ期精巢, ×400;
7. 1 时相早期卵原细胞, ×200;
8. 1 时相晚期卵原细胞, ×400;
9. 2 时相早期初级卵母细胞, ×400;
10. 2 时相晚期初级卵母细胞, ×400。

Explaination of Plate Ⅰ

1. Sex differentiation early stage ovarian, gonium proliferation, ×400;
2. Stage Ⅰ testis, ×400;
3. Stage Ⅱ testis, ×400;
4. Stage Ⅲ testes, ×400
5. Stage Ⅳ testes, ×400
6. Stage Ⅴ testes, ×400
7. Early phase 1 oogonia, ×200;
8. Late phase 1 oogonia, ×400;
9. Early phase 2 primary oocyte, ×400;
10. Late phase 2 primary oocyte, ×400.



图版 I Plate I

（图版说明见文末 Explaination of Plate I at the end of the text）