

吳郭魚第一型及第三型肝細胞核因子於繁殖系統之調控研究

余浩成、黃尉東

E-mail: 9417454@mail.dyu.edu.tw

摘要

吳郭魚為淡水養殖魚類中最普遍之魚種，且其具成長快速及抗病力強之特性，故為魚類養殖相關研究之重要模式魚種。而肝細胞核因子(hepatocyte nuclear factors, HNFs)為富含於肝臟中之轉錄因子，其能活化組織專一性之基因表現，並與胚胎之生長與發育極具相關性。而本研究室先前於吳郭魚之性腺發現肝細胞核因子之存在，且其表現可受固醇類荷爾蒙所調控，因此推測吳郭魚之繁殖系統可能存在另一異於傳統之內分泌路徑。為探討吳郭魚肝細胞核因子於繁殖系統之調控作用，本研究以性成熟之吳郭魚性腺進行體外(*in vitro*)試驗，先將性腺以未添加固醇類荷爾蒙之培養液預培養6小時後，續分別添加0, 0.1, 1, 10, 100及1000 nM等6組不同濃度之 β -estradiol及hydrocortisone再培養6小時，經抽取其total RNA進行反轉錄?-聚合?連鎖反應(reverse transcriptase-polymerase chain reaction, RT-PCR)後以半定量(semi-quantitative)方式分析HNFs於各組之表現，另以添加類固醇後0, 6, 12, 18, 24, 30及36 h等7組時間點做相同之分析。結果顯示，HNF1- α 及HNF3- β 之表現並未受前述兩者固醇類荷爾蒙之影響，但HNF3- β 之表現量則與荷爾蒙劑量呈正相關之趨勢，且以 β -estradiol作用較佳，其最佳作用濃度為10 nM，並於作用12 h後HNF3- β 之表現量可達至高原期。另以免疫組織化學法(immunohistochemistry)偵測1周、2周及1月齡之吳郭仔稚魚體內肝細胞核因子之表現，結果顯示，於所觀察之不同年齡之魚體內皆可偵測HNF-1 α 、HNF-1 β 及HNF-3 β 在肝臟及其他消化道上皮組織中表現。由上述之結果証實，吳郭魚性腺中之HNF-3 β 表現可受固醇類荷爾蒙所調控，並可能參與性腺之發育及配子生成，而HNFs是否於幼魚階段即對其生長及發育有所影響，仍需試驗證實之。

關鍵詞：吳郭魚；性腺；肝細胞核因子；固醇類荷爾蒙

目錄

目錄 封面內頁 簽名頁 授權書.....	iii 中文摘要.....
iv 英文摘要.....	vi 謝誌.....
viii 目錄.....	ix 圖目錄.....
xii 表目錄.....	xiv 壹、前言.....
1 貳、文獻檢討.....	3 一、吳郭魚之簡介.....
3 (一)、種類、形態、生態及成長特性.....	3 (二)、特殊生活行為特殊生活行為..... 5
二、繁殖系統之概論.....	5 (一)、雌魚性腺之內分泌..... 6 (二)、雄魚性腺之內分泌.....
魚性腺之內分泌.....	7 三、類胰島素生長因子(Insulin-like growth factors, IGFs)..... 9 (一)、第一型類胰島素生長因子(IGF-1)..... 10 (二)、第二型類胰島素生長因子(IGF-2)..... 11 (三)、生長激素-類胰島素生長因子軸(GH-IGF axis).... 12 四、肝細胞核因子(Hepatocyte nuclear factors, HNFs)..... 13 (一)、第一型肝細胞核因子(HNF-1)..... 14 (二)、第三型肝細胞核因子(HNF-3)..... 15 五、HNF與IGF之相關研究.....
16 參、材料與方法.....	18 一、實驗材料.....
18 (一)、試驗動物.....	18 二、實驗方法.....
18 (一)、性腺組織之體外培養.....	18 1. 劑量相關(Dose dependent)試驗..... 19 2. 時間相關(Time dependent)試驗.....
19 (二)、Total RNA之分離.....	19 (三)、反轉錄?-聚合?連鎖反應(Reverse transcriptase polymerase chain reaction, RT-PCR)..... 20 (四)、PCR雜合反應(PCR-hybridization).....
22 (五)、免疫組織化學法(Immunohistochemistry).....	23 1. 組織之石蠟包埋及切片.....
23 2. 免疫組織化學分析.....	24 (六)、統計分析..... 25 肆、結果.....
26 一、Beta-estradiol對吳郭魚性腺中肝細胞核因子表現之影響.....	26 二、Hydrocortisone對吳郭魚性腺中肝細胞核因子表現之影響.....
26 三、不同濃度之 β -estradiol對HNF-3 β 於吳郭魚性腺中表現之影響.....	26 三、不同濃度之 β -estradiol對HNF-3 β 於吳郭魚性腺中表現之影響.....
27 四、不同濃度之hydrocortisone對HNF-3 β 於吳郭魚性腺中表現之影響.....	27 四、不同濃度之hydrocortisone對HNF-3 β 於吳郭魚性腺中表現之影響.....
27 五、Beta-estradiol於不同作用時間對HNF-3 β 於吳郭魚性腺中表現之影響.....	27 五、Beta-estradiol於不同作用時間對HNF-3 β 於吳郭魚性腺中表現之影響.....
28 六、Hydrocortisone於不同作用時間對HNF-3 β 於吳郭魚性腺中表現之影響.....	28 六、Hydrocortisone於不同作用時間對HNF-3 β 於吳郭魚性腺中表現之影響..... 29 七、肝細胞核因子於吳郭仔稚魚全身之表現..... 30 伍、討論..... 31 陸、結論..... 35 參考文獻.....
54 圖 目 錄 圖一、實驗流程圖.....	36 圖二、初代培養所使用之吳郭魚性腺...

..... 37 圖三、不同濃度 -estradiol對吳郭魚性腺之肝細胞核因子表現之影響.....	
..... 38 圖四、不同濃度hydrocortisone對吳郭魚性腺之肝細胞核因子表現之影響.....	
..... 39 圖五、不同濃度之 -estradiol對吳郭魚睺丸中HNF-3 表現之影響.....	
..... 40 圖六、不同濃度之 -estradiol對吳郭魚濾泡中HNF-3 表現之影響.....	
..... 41 圖七、不同濃度之hydrocortisone對吳郭魚睺丸中HNF-3 表現之影響.....	
..... 42 圖八、不同濃度之hydrocortisone對吳郭魚濾泡中HNF-3 表現之影響.....	
43 圖九、Beta -estradiol培養時間對吳郭魚睺丸中HNF-3 表現之影響.....	44 圖
十、Beta -estradiol培養時間對吳郭魚濾泡中HNF-3 表現之影響.....	45 圖
一、Hydrocortisone培養時間對吳郭魚睺丸中HNF-3 表現之影響.....	46 圖十二
、Hydrocortisone培養時間對吳郭魚濾泡中HNF-3 表現之影響.....	47 圖十三、免疫組
織化學法偵測HNF-1?於1週、2週及1月齡吳郭仔稚魚全身之表現.....	48 圖十四、免疫組織化學法偵
測HNF-1 於1週、2週及1月齡吳郭仔稚魚全身之表現.....	49 圖十五、免疫組織化學法偵測HNF-3
於1週、2週及1月齡吳郭仔稚魚全身之表現.....	50 圖十六、免疫組織化學法偵測未添加初級抗體
於1週、2週及1月齡吳郭仔稚魚全身之表現.....	51 圖十七、傳統內分泌路徑與固醇類荷爾蒙-肝細胞核因
子-類胰島素因子-性腺路徑假說.....	52 表目錄表一、本試驗各因子之專一引子序列.....
..... 53	

參考文獻

- 沈世傑，1990。台灣魚類檢索。南天書局。pp. 321-327。邵廣昭，1996。台灣常見魚貝類圖說。台灣省漁業局。pp. 125-146。高承志，1996。魚類生殖的內分泌基礎。漁業推廣。117: 15-23。施稼芳，1994。魚類生理學。水產出版社。pp. 318-321。Allan, G., Flint, D. and Patel, K. 2001. Isulin-like growth factor axis during embryonic development. *J. Reprod. Fertil.* 122: 31-39. Baker, J. P., Robertson, E. J. and Efstratiadis, A. 1993. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* 75: 73-82. Baker, J., Hardy, M. P., Zhou, J., Bondy, C., Lupu, F., Bellve, A. R. and Efstratiadis, A. 1996. Effects of an Igf 1 gene null mutation on mouse reproduction. *Mol. Endocrinol.* 10: 903-918. Bell, G. I., Gerhard, D. S., Fong, N. M., Sanches-Pescador, R. and Rall, L. B. 1985. Isolation of the human insulin-like growth factor gene: Insulin-like growth factor II and Isulin genes are contiguous. *Proc. Natl. Acad. Sci.* 82: 6450-6454. Berger, R. R. and Sanders, M. 2000. Estrogen modulates HNF-3 mRNA levels in the developing chick oviduct. *DNA Cell Biol.* 19: 103-112. Bigsby, R. M. and Li, A. 1994. Differentially regulated immediate early genes in the rat uterus. *Endocrinology* 134: 1820-6. Billard, R., Fostier, A., Weil, C. and Breton, B. 1982. Endocrine control of spermatogenesis in teleost fish. *Can. J. Fish Aquat. Sci.* 39: 65-79. Binkley, S. A. 1995. Growth hormone from anterior pituitary. In "Endocrinology". Ed. Binkley, S. A. Harpercollins College Div. pp. 107-126. Cao, Q. P., Duguay, S. J., Plisetskaya, E., Steiner, D. F. and Chan, S. J. 1989. Nucleotide sequence and growth hormone-regulated expression of salmon insulin-like growth factor I mRNA. *Mol. Endocrinol.* 3: 2005-2010. Cereghini, S., Ott, M. O., Power, S. and Maury, M. 1992. Expression patterns of vHNF-1 and HNF-1 homeoproteins in early postimplantation embryos suggest distinct and sequential developmental roles. *Development* 116: 783-797. Chang, C. F. and Yueh, W. S. 1990. Annual cycle of gonadal histology and steroid profiles in the juvenile males and adult females of the protandrous black porgy, *Acanthopagrus schlegeli*. *Aquaculture* 91: 179-196. Chang, C. F., Lau, E. L. and Lin, B. Y. 1995. Estradiol-17 β suppresses testicular development and stimulates sex reversal in protandrous black porgy (*Acanthopagrus schlegeli*). *Fish Physiol. Biochem.* 14: 481-488. Chen, J. Y., Tsai, H. L., Chang, C. Y., Wang, J. I., Shen, S. C. and Wu, J. L. 1998. Isolation and characterization of tilapia (*Oreochromis mossambicus*) insulin-like growth factors gene and proximal promoter region. *DNA Cell Biol.* 17: 359-376. Cirillo, L. A., McPherson, C. E., Bossard, P., Stevens, K., Cherian, S., Shim, E. Y., Clark, K. L., Burley, S. K. and Zaret, K. S. 1998. Binding of the winged-helix transcription factor HNF3 to a linker histone site on the nucleosome. *EMBO J.* 17: 244-254. Clark, K. L., Halay, E. D., Lai, E. and Burley, S. K. 1993. Co-crystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. *Nature* 364: 412-420. Cohick, W. S. and Clemmons, D. R. 1993. The insulin-like growth factors. *Annu. Rev. Physiol.* 55: 131-153. De Silva, S. S. and Sirisena, H. K. G. 1988. Observations on the nesting habits of *Oreochromis mossambicus* (Peters) (Pisces:Cichlidae) in Sri Lankan reservoirs. *J. Fish Biol.* 33: 689-696. Deryckere, F., McNair, A. and Gannon, F. 1996. Hepatocyte nuclear factor 4 (HNF4) binding sites in the salmon HNF1 promoter. *Gene* 175: 35-41. Duan, C. 1997. The insulin-like growth factor system and its biological action in fish. *Am. Zool.* 37: 491-503. Duguay, S. J., Swanson, P. and Dickhoff, W. W. 1994. Differential expression and hormonal regulation of alternatively spliced IGF-I mRNA transcripts in salmon. *J. Mol. Endocrinol.* 12: 25-37. Duguay, S. J., Lai-Zhang, J., Steiner, D. F., Funkenstein, B. and Chan, S. J. 1996. Developmental and tissue-regulated expression of IGF-I and IGF-II mRNAs in *Sparus aurata*. *J. Mol. Endocrinol.* 16: 123-132. Froesch, E. R., Schmid, C., Schwander, J. and Zapf, J. 1985. Actions of insulin-like growth factors. *Annu. Rev. Physiol.* 47: 443-467. Greene, M. W. and Chen, T. T. 1999. Quantitation of IGF-I, IGF-II, and multiple insulin receptor family member messenger RNAs during embryonic development in rainbow trout. *Mol. Reprod. Dev.* 54: 348-361. Hayashi, Y., Wang, W., Ninomiya, T., Ohta, K. and Itoh, H. 1999. Liver enriched transcription factor and differentiation of hepatocellular carcinoma. *Mol. Pathol.* 52: 19-24. Hines, G. A., Boots, L. R., Wibbels, T. and Watts, S. A. 1999. Steroid levels and steroid metabolism in relation to early gonadal development in the tilapia (*Oreochromis niloticus*). *Gen. Comp. Endocrinol.* 114: 235-248. Hirose, K., Adachi, S. and Nagahama, Y. 1985. Changes in plasma steroid hormone levels

during sexual maturation in the ayu *Plecoglossus altivelis*. Bull. Jpn. Soc. Sci. Fish. 51: 399-403. Holly, J. M. and Wass, J. A. 1989. Insulin-like growth factors autocrine, paracrine or endocrine ? New perspectives of the somatomedin hypothesis in light of recent developments. J. Endocrinol. 122: 611-618. Huang, W. T., Gong, H. Y., Lin, C. F., Weng, C. F., Chen, M. C. and Wu, J. L. 2001. Hepatocyte nuclear factors-1, -1, and 3, expressed in the gonad of tilapia (*Oreochromis mossambicus*). Biochem. Biophys. Res. Comm. 288: 833-840. Huang, W. T., Yu, H. C., Yang, H. C., Lin, C. J. F., Gong, H. Y., Weng, C. F. and Wu, J. L. 2004. Studies on the expression and localization of hepatocyte nuclear factors (HNFs) in the gonads of tilapia (*Oreochromis mossambicus*). In " Proc. Symp. of the 5th Congress of Asian and Oceanian Society for Comparative Endocrinology ", Nara, Japan. P-054. Hull, K. L. and Harvey, S. 2001. Growth hormone: roles in female reproduction. J. Endocrinol. 168: 1-23. Imae, M., Inoue, Y., Fu, Z., Kato, H. and Noguchi, T. 2000. Gene expression of the three members of hepatocyte nuclear factor-3 is differentially regulated by nutritional and hormonal factors. J. Endocrinol. 167: 1-5. Kaestner, K. H. 2000. The hepatocyte nuclear factor 3 (HNF3 or FOXA) family in metabolism. Trends Endocrinol. Metab. 11: 281-285. Katsu, Y., Yamashita, M. and Nagahama, Y. 1999. Translational regulation of cyclin B mRNA by 17alpha, 20beta-dihydroxy-4-pregnen-3-one (maturation-inducing hormone) during oocyte maturation in a teleost fish, the goldfish (*Carassius auratus*). Mol. Cell. Endocrinol. 158: 79-85. Kaufmann, E. and Knochel, W. 1996. Review article Five years on the wings of fork head. Mech. Dev. 57: 3-20. Kavsan, V. M., Koval, A. P., Grebenjuk, V. A., Chan, S. J., Steiner, D. F., Roberts, C. T. Jr and Le Roith, D. 1993. Structure of the chum salmon insulin-like growth factor I gene. DNA Cell Biol. 12: 729-737. Kermouni, A., Mahmoud, S. S., Wang, S., Moloney, M. and Habibi, H. R. 1998. Cloning of a full-length insulin-like growth factor-I complementary DNA in the goldfish liver and ovary and development of a quantitative PCR method for its measurement. Gen. Comp. Endocrinol. 111: 51-60. Khan, I. A. and Thomas, P. 1999. Ovarian cycle, teleost fish. In " Encyclopedia of reproduction ". Eds. Knobil, E. and Neill, J. D. Academic Press. pp. 552-564. Kim, S. J., Ogasawara, K., Park, J. G., Takemura, A. and Nakamura, M. 2002. Sequence and expression of androgen receptor and estrogen receptor gene in the sex types of protogynous wrasse, *Halichoeres trimaculatus*. Gen. Comp. Endocrinol. 127: 165-173. Kulik, V. P., Kavsan, V. M., Schaik, F. M., Nolten, L. A., Steenbergh, P. H. and Sussenbach, J. S. 1995. The promoter of the salmon insulin-like growth factor I gene is activated by hepatocyte nuclear factor 1. J. Biol. Chem. 270: 1068-1073. Lai, E., Prezioso, V. R., Smith, E., Litvin, O., Costa, R. H. and Darnell, Jr., J. E. 1990. HNF-3, a hepatocyte-enriched transcription factor of novel structure is regulated transcriptionally. Genes Dev. 4: 1427-1436. Le Gac, F., Loir, M., le Bail, P.Y. and Ollitrault, M. 1996. Insulin-like growth factor (IGF-I) mRNA and IGF-I receptor in trout testis and in isolated spermatogenic and Sertoli cells. Mol. Reprod. Dev. 44: 23-35. Le Menn, F. and Burzawa-Gerard, E. 1985. Effect of carp gonadotropin (cGTH) and a fraction unabsorbed on concanavalin A-Sepharose obtained from cGTH on vitellogenesis in the hypophysectomized marine teleost *Gobius niger*. Gen. Comp. Endocrinol. 57: 23-36. Matsubara, T., Adachi, S., Ijiri, S. and Yamauchi, K., 1995. Changes of lipovitellin during in vitro oocyte maturation in Japanese flounder *Paralichthys olivaceus*. Fish. Sci. 61: 478-481. McCormick, S.D., Sakamoto, T., Hasegawa, S. and Hirano, T. 1991. Osmoregulatory actions of insulin-like growth factor-I in rainbow trout (*Oncorhynchus mykiss*). J. Endocrinol. 130: 87-92. Meton, I., Boot, E. P. J., Sussenbach, J. S. and Steenbergh, P. H. 1999. Growth hormone induces insulin-like growth factor-1 gene transcription by a synergistic action of STAT5 and HNF-1. FEBS Lett. 444: 155-159. Mirua, T. 1996. Molecular control mechanisms on spermatogenesis in fish. Nippon Suisan Gakkaishi 62: 547-550. Moriyama, S., Ayson, F. G. and Kawauchi, H. 2000. Growth regulation by insulin-like growth factor-I in fish. Biosci. Biotechnol. Biochem. 64: 1553-1562. Moyle, P. B. and Cech, J. J. 1996. Fishes: " An Introduction to Ichthyology. " 3rd ed. Eds. Moyle, P. B. and Cech, J. J. Prentice Hall. pp. 137-171. Murphy, L. J., Bell, G. I., Duckworth, M. L. and Friesen, H. G. 1987. Identification, characterization, and regulation of a rat complementary deoxyribonucleic acid which encodes insulin-like growth factor-I. Endocrinology 121: 684-691. Nagahama, Y. 1997. 17, 20-dihydroxy-4-pregnen-3-one, a maturation-inducing hormone in fish oocytes: Mechanisms of synthesis and action. Steroids 62: 190-196. Oshiro, T. and Hibiya, T. 1981. Relationship of yolk globules fusion to oocyte water absorption in the plaice, *Limanda yokohamae* during meiotic maturation. Bull. Jpn. Soc. Sci. Fish. 47: 1123-1130. Ott, M. O., Rey, C. J., Cereghini, S. and Yaniv, M. 1991. vHNF1 is expressed in epithelial cells of distinct embryonic origin during development and precedes HNF1 expression. Mech. Dev. 36: 47-58. Oyadomari, S., Matsuno, F., Chowdhury, S., Kimura, T., Iwase, K., Araki, E., Shichiri, M., Mori, M. and Takiguchi, M. 2000. The gene for hepatocyte nuclear factor (HNF)-4alpha is activated by glucocorticoids and glucagon, and repressed by insulin in rat liver. FEBS Lett. 478: 141-146. Palamarchuk, A., Gritsenko, O., Holthuizen, E., Sussenbach, J., Caelers, A., Reinecke, M. and Kavsan, V. 2002. Complete nucleotide sequence of the chum salmon insulin-like growth factor II gene. Gene 295: 223-230. Palamarchuk, A. Y., Kavsan, V. M., Sussenbach, J. S. and Holthuizen, P. E. 1999. The chum salmon IGF-II gene promoter is activated by hepatocyte nuclear factor 3. FEBS Lett. 446: 251-255. Perez-Sanchez, J., Weil, C. and Lebail, P. Y. 1992. Effects of human insulin-like growth factor-I on release of growth hormone by rainbow trout (*Oncorhynchus mykiss*) pituitary cells. J. Exp. Zool. 262: 287-290. Peter, R. E., Trudeau, V. L. and Sloley, B. D. 1991. Brain regulation of reproduction in teleosts. Bull. Inst. Zool. Acad. Sin. 16: 89-118. Pinter, J. and Thomas, P. 1999. Induction of ovulation of mature oocytes by the maturation-inducing steroid 17, 20, 21-trihydroxy-4-pregnen-3-one in the spotted seatrout. Gen. Comp. Endocrinol. 115: 200-209. Reber, M. and Cereghini, S. 2001. Variant hepatocyte nuclear factor 1 expression in the mouse genital tract. Mech. Dev. 100: 75-78. , K. L., Halay, E. D., Lai, E. and Burley, S. K. 1993. Cocrystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. Nature 364: 412-420. Riley, L. G., Hirano, T. and Grau, E. G. 2004. Estradiol-17beta and dihydrotestosterone differentially regulate vitellogenin and insulin-like growth factor-I production in primary hepatocytes of the tilapia *Oreochromis mossambicus*. Comp. Biochem. Physiol. 138: 177-186. Rinkerknecht, E. and Humbel, R. E. 1978. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. J. Biol. Chem. 253: 2769-2776. Rotwein, P., Pollock, K. M., Didier, D. K. and Krivi, G. G.. 1986. Organization and sequence of the human insulin-like growth factor I gene. Alternative RNA

processing produces two insulin-like growth factor I precursor peptides. J. Biol. Chem. 261: 4828-4832. Rouiller-Fabre, V., Lecref, L., Gautier, C., Saez, J. M. and Habert, R. 1998. Expression and effect of insulin-like growth factor I on rat fetal Leydig cell function and differentiation. Endocrinology 139: 2926-2934. Sakai, N., Iwamatsu, T., Yamauchi, K. and Nagahama, Y. 1987. Development of the steroidogenic capacity of medaka (*Oryzias latipes*) ovarian follicles during vitellogenesis and oocyte maturation. Gen. Comp. Endocrinol. 66: 333-342. Sakai, N., Ueda, H., Suzuki, N. and Nagahama, Y. 1989. Steroid production by amago salmo (*Oncorhynchus rhodurus*) testes at different developmental stages. Gen. Comp. Endocrinol. 75: 231-240. Sakamoto, T. and Hirano, T. 1993. Expression of insulin-like growth factor I gene in osmoregulatory organs during seawater adaptation of the salmonid fish: possible mode of osmoregulatory action of growth hormone. Proc. Natl. Acad. Sci. 90: 1912-1916. Shimatsu, A. and Rotwein, P. 1987. Mosaic evolution of the insulin -like growth factors. Organization, sequence, and expression of the rat insulin-like growth factor I gene. J. Biol. Chem. 262: 7894-7900. Simpson, E. R. and Waterman, M. R. 1988. Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. Annu. Rev. Physiol. 50: 427-440. Smit, L. S., Meyer, D. J., Billestrup, N., Norstedt, G., Schwartz, J. and Carter-Su, C. 1996. The role of the growth hormone (GH) receptor and JAK1 and JAK2 kinases in theactivation of Stats 1, 3, and 5 by GH. Mol. Endocrinol. 10: 519-533. Sourdive, D. J. D. and Yaniv, M. 1997. The hepatic nuclear factor1 . In " Transcription factors in eukaryotes. " Ed. Paparassiliou, A. G. Landes Bioscience. pp. 189-209. Spiliotis, B. E. 2003. Growth hormone insufficiency and its impact on ovarian function. Ann. NY Acad. Sci. 997: 77-84. Stenson, C., McNair, A., Byrnes, L., Murphy, M., Smith, T. and Gannon, F. 2000. Atlantic salmon HNF-3/forkhead: cDNA sequence, evolution, expression, and functional analysis. DNA Cell Biol. 19: 59-68. Stenson, C., McNair, A., Curley, M., Smith, T. and Gannon, F. 2002. A role for HNF-3 in the regulation of the HNF-1 gene of the Atlantic salmon. Mol. Genet. Genomics 266: 832-837. Ueda, H., Kambegawa, A. and Nagahama, Y. 1984. In vitro 11-keto- teatosterone and 17?, 20?-dihydroxy-4-pregnen-3-one production by testicular fragments and isolated sperm of rainbow trout, *Salmo gairdneri*. J. Exp. Zool. 231: 435-439. Ueda, H., Nagahama, Y., Tashiro, F. and Crime, L. W. 1983. Some endocrine aspects of precocious sexual maturation in the amago salmon, *Oncorhynchus rhodurus*. Bull. Jpn. Soc. Sci. Fish 49: 587-596. Wang, J., Stromstedt, P., O ' brien, R. and Granner, D. 1996. Hepatic nuclear factor 3 is an accessory factor required for the stimulation of phosphoenolpyruvate carboxykinase gene transcription by glucocorticoids. Mol. Endocrinol. 10: 794-800. Weigel, D., Jurgens, G., Kuttner, F., Seifert, E. and Jackle, H. 1989. The homeotic gene fork head encodes a nuclear protein and is expressed in the terminal regions of the *Drosophila* embryo. Cell 57: 645-658. Yoshikuni, M. and Nagahama, Y. 1991. Endocrine regulation of gametogenesis in fish. Bull. Inst. Zool. Acad. Sin. 16: 139-172.