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# GONADOTROPIN SPECIFICITY AND SPECIES DIVERSITY OF GONADAL STEROID HORMONE FORMATION IN FISH

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**J. Y.-L. Yu, S.-T. Shen, Y.-C. Wu, S.-H. Chen and C.-T. Liu** (1991) Gonadotropin specificity and species diversity of gonadal steroid hormone formation in fish. *Bull. Inst. Zool., Academia Sinica, Monograph* 16: 61-88. Gonadotropin preparations from four classes of tetrapods (amphibian, avian, reptilian and mammalian) and several teleost fish were employed to investigate the species specificity and gonadotropin (LH/FSH) specificity of *in vitro* steroid formation by teleost testes or ovaries. The present study included the testes of common carp, *Cyprinus carpio* (Order Cypriniformes); tilapia, *Tilapia mossambica*×*T. nilotica* (Order Perciformes); swamp eel, *Fluta alba* (Order Synbranchiformes); and ovaries of common carp, black silver carp, *Aristichythys nobilis* (Order Cypriniformes) and rainbow trout, *Salmo gairdneri* (Order Salmoniformes). Testicular testosterone formation in teleost testes exhibits a great diversity in gonadotropin specificity. Testes from each of the three teleost orders represented in this study show a distinct specificity to avian LHs; while avian FSHs are inactive. Tilapia and swamp eel testes exhibit high specificity to mammalian (ovine) LH and placental gonadotropin (hCG), while common carp testis is unresponsive to ovine LH and hCG. *Cyprinus carpio* testis is not responsive to LHs and FSHs from reptilian (sea turtle) and amphibian (bullfrog). In these testicular assay systems, mammalian (ovine) FSH is inactive in *Cyprinus carpio*, slightly active in *Fluta alba*, but rather active in tilapia (*Tilapia mossambica*×*T. nilotica*), although being less potent as compared to ovine LH. Gonadotropin specificity of estradiol-17$\beta$ formation responses of ovaries from common carp is very similar to that observed for common carp testis. Ovine LH and hCG are inactive in rainbow trout ovaries.

Comparison of the relative potencies of gonadotropins from different tetrapods and teleosts in teleost testis and ovary bioassays

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revealed that: 1) tremendous variability exists in the potencies of tetrapod LHs when assayed in either testis or ovary from a single species of teleost; and 2) a great degree of interspecific difference exists in either testis or ovary among teleosts in response to tetrapod LHs. Consequently, no clear correlation with the phylogenic relationship is observed with respect to evolution of LH molecules among tetrapod vertebrates studied. The present study also demonstrated that the gonads from all five teleost species examined are highly responsive to stimulation with silver carp GTH, implicating that the phylogenic resemblance during evolutionary process exists in both GTH molecules and gonadal receptors among the five teleost species studied. The observations that avian LHs are highly active in stimulating gonadal steroidogenesis *in vitro* in most of the teleosts examined, provide a basis for further studies of these hormones for potential applications in controlled reproduction in aquaculture.

**Key words:** Tetrapod and teleost gonadotropins, *In vitro* formation of steroid hormones, Cyclic AMP, Gonadotropin specificity, Species diversity.

All vertebrate species are characteristic of possession of a pituitary gland which synthesizes and secretes gonadotropin (GTH). In mammals, birds, reptiles and amphibians, there are two distinct types of GTH, luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Licht *et al.*, 1977; Pierce and Parsons, 1981). In fish, although two types of GTHs (GTH-I and GTH-II) are found in some teleosts, neither of them is structurally identical to LH or FSH (Kawauchi *et al.*, 1987, 1989; Suzuki *et al.*, 1988a, 1988b). All gonadotropins are composed of two subunits: $\alpha$-subunits are common between LH and FSH, while $\beta$-subunits are specific and determine biological activity of the hormone.

One of the physiological functions of GTH is to stimulate steroidogenesis in testis and ovary. Comparative studies have demonstrated that a high degree of variability exists among tetrapod vertebrates in the hormonal specificity of gonadotropin regulation of testicular androgen formation (Licht *et al.*, 1977; Licht, 1986). In mammals and birds, distinct LH-specificity exists in their testicular androgen formation (Licht 1986; Yu and Wang, 1987). Although excessive amounts of FSHs are also effective, it is virtually due to LH impurities contained in FSH preparations (Yu and Wang, 1987). In reptiles, some species show a general lack of

gonadotropin specificity, while other species exhibit high LH specificity (Licht and Pearson, 1969; Tsui and Licht. 1977; Licht and Papkoff, 1985; Licht, 1986; Yu *et al.*, 1991). Most of amphibian species studied exhibit high LH-specificity (Licht and Papkoff, 1974; Muller, 1977; Muller and Licht, 1979; Yu *et al.*, 1991).

In fish, for a long time, only a single gonadotropin was isolated (Sumpter *et al.*, 1978; Breton, 1981; Burzawa-Gerard, 1982; Kobayashi *et al.*, 1985; Goos *et al.*, 1986; Banerjee *et al.*, 1989b); although existences of isohormones were observed in several teleost apecies (Huang *et al.*, 1981; Ando and Ishii, 1988; Yu and Shen, 1989; Banerjee *et al.*, 1989a), and immunocytochemical evidence for one or two pitutary GTH cell types in several teleost species was controversial (Van Oordt and Peute, 1983). The teleost GTH is homologous to mammalian LH and FSH, and has been thought to control all aspects of gonadal development and functions. However, a second GTH, the vitellogenic GTH, was found in pituitaries of several teleost species (Idler and Ng, 1979, 1983). The vitellogenic GTH appears to be chemically different from the "classical" teleost GTH (maturational GTH) which is chemically and functionally related to mammalian LH and FSH. Kawauchi and coworkers (Kawauchi *et al.*, 1987; Suzuki *et al.*, 1988a; Kawauchi *et al.*, 1989) further isolated two molecular forms of gonadotropin-like glycoproteins: GTH-I and GTH-II; the two distinct GTHs which are chemically homologous to LH or FSH, and show similar biological activities both *in vivo* and *in vitro*. However, neither of them is identical to LH or FSH in term of physicochemical and functional properties. Thus, studies on testicular androgen formation in fish responding to piscine gonadotropins do not lead to illustrating the issue of LH/FSH specificity. Consequently, investigations were made to explore the gonadotropin specificity in teleosts by observation of the *in vitro* androgen formation response by teleost testes to LH and FSH preparations purified from selective species of the tetrapod vertebrate classes (Bona-Gallo and Licht, 1981; Yu and Lin, 1985). The results indicate that a high degree of variability exist in both gonadotropin specificity and species specificity of the teleost testes. Information on the corresponding specificities in estrogen formation by teleost ovary is even less than those of teleost testicular formation. Bona-Gallo and

Licht (1983) compared the gonadotropin specificity of estrogen by ovaries from three different species and demonstrated that the specificity of the ovarian steroidogenic response to gonadotropins parallels that observed for testosterone secretion in the males of the same three species of fish, but the differential actions of the tetrapod hormones are more exaggerated for the testes.

Since only few species of teleosts were examined so far, we thus conducted studies to extend information on the hormonal specificity and species specificity of steroidogenesis in teleosts by measuring the responsiveness of testicular testosterone and ovarian estradiol-17$\beta$ formations *in vitro*, to diverse tetrapod and piscine gonadotropins.

## MATERIALS AND METHODS

### Hormones and chemicals

Ovine LH, Ovine FSH, and human chorionic gonadotropin (hCG) were supplied by the National Hormone and Pituitary Program, NIADDK, Baltimore, MD, USA. Bovine and porcine gonadotropins were from the USDA Animal Hormone Program. Chicken LH and FSH, and bullfrog LH and FSH from Dr. Susumu Ishii (Adachi *et al.*, 1979; Sakai and Ishii, 1980; Ishii and Furuya, 1981), and gonadotropins from turkey, ostrich, and sea turtle were supplied by Dr. H. Papkoff (Papkoff *et al.*, 1976, 1982; Licht and Papkoff, 1985). Gonadotropins from duck, goose, grass carp, silver carp and loach were purified by our laboratory (Yu *et al.*, 1987; Yu and Shen, 1989; Banerjee *et al.*, 1989a). Medium 199 (with Hanks' Salts and L-glutamine) was from GIBCO Laboratories, Chagrin Falls, Ohio. HEPES (N-2-hydroxy-ethyl piperazine N-2-ethane sulfonic acid), MIX (1-methyl-3-isobutyl-xanthine), bovine serum albumin (BSA, Fraction V), penicillin and streptomycin were purchased from Sigma Chemical Co., St. Louis, MO. The specifications and sources of the hormones assayed in the present study are summarized in Table 1.

### Animals

Fish were purchased from a local commercial supplier. The body weights and gonadal weights of teleosts used in the present study are indicated in Table 2.

### Testicular incubations and Testosterone radioimmunoassay

The procedures of *in vitro* incubation of minced testicular tissue

Table 1
The specification and source of gonadotropins used in the present study

| Vertebrate Class | Hormones | Code no. | Source |
|---|---|---|---|
| Mammalian | ovine LH | NIADDK-oLH-25 | NIH |
| | bovine LH | USDA-bLH-B-1 | USDA |
| | porcine LH | USDA-pLH-B-1 | USDA |
| | hCG | hCG CR-125 | NIH |
| | ovine FSH | NIADDK-oFSH-17 | NIH |
| | porcine FSH | NIADDK-pFSH-B1 | USDA |
| Avian | chicken LH | CANOMS124326 | S. Ishii |
| | chicken FSH | AGCQSQ123445C | S. Ishii |
| | turkey LH | B25B | H. Papkoff |
| | turkey FSH | B150A | H. Papkoff |
| | ostrich LH | SC6BRB | H. Papkoff |
| | ostrich FSH | SC31RB | H. Papkoff |
| | duck LH | d-C2D1G | JYL. Yu |
| | goose LH | g-C2D1G | JYL. Yu |
| Reptilian | sea turtle LH | TC76D | H. Papkoff |
| | sea turtle FSH | TC138 | H. Papkoff |
| Amphibian | bullfrog LH | FL451B (F-LH-II) | S. Ishii |
| | bullfrog FSH | FF1361B (F-FSH-III) | S. Ishii |
| Piscine | silver carp GTH | 37K-scGTH | JYL. Yu |
| | | 43K-scGTH | JYL. Yu |
| | grass carp GTH | gcGTH-DEI | JYL. Yu |
| | loach GTH | l-C2D1G | JYL. Yu |

for testosterone formation of fish were similar to those described previously (Yu and Lin, 1986). Fish were sacrificed by decapitation and their testes were removed and transferred to glass petri dish containing ice-cold Hanks' balanced Salts Solution (HBSS, pH 7.40). Testes were cut by surgical scissors into fine pieces, and washed with HBSS several times. The sedimented testicular tissues were then stirred with a magnetic stirrer in incubation medium (Medium 199 containing 0.125 mM MIX, Hanks' salts, L-glutamine, 25 mM HEPES, penicillin 10,000 units/100 ml, streptomycine 5 mg/100 ml, 0.1% BSA, 0.1% sodium bicarbonate, pH 7.40). Aliquots of 0.5 ml tissue suspension were pipetted into each of incubation vials that contained 0.1 ml of various doses of gonadotropins and 0.9 ml incubation medium. Incubation was conducted

Table 2

The body weights, gonadal weights and gonadosomatic index (GSI) of teleosts used in the present study

| Order | Cypriniformes | | | Perciformes | Synbranchiformes | Salmoniformes |
|---|---|---|---|---|---|---|
| Family | Cyprinid | | | Cichlid | Synbranchid | Salmonid |
| Species | *Cyprinus carpio* | | *Aristichythys nobilis* | *T. mossambica*× *T. nilotica* | *Fluta alba* | *Salmo gairdneri* |
| (common name) | (common carp) | | (black silver carp) | (tilapia) | (swamp eel) | (rainbow trout) |
| | male | female | female | male | male | female |
| Body Wt[a] (g) | 705 | 649 | 1,643 | 577 | 280 | 493 |
| | (436–1,210) | (474–1,010) | (1,500–1,739) | (486–624) | (250–300) | (430–570) |
| Gonad Wt[a] (g) | 110 | 140 | 77 | 4.03 | 0.88 | 1.41 |
| | (48–162) | (52–236) | (60–100) | (1.31–5.60) | (0.70–1.00) | (0.89–2.00) |
| GSI (%)[a] | 16.8 | 20.8 | 4.5 | 4.67 | 0.30 | 0.29 |
| | (11–26) | (10.3–30) | (4.0–7.0) | (0.20–0.89) | (0.20–0.50) | (0.21–0.42) |
| No. of Assays[b] | 5 | 4 | 2 | 3 | 2 | 2 |

a: Mean and range.

b: In each assay, one to three fish were used.

at 25°C for 4 h, under continuous aeration of 95% $O_2$ and 5% $CO_2$ in a Dubnoff incubator shaken at 100 cycles/min. Following incubation, the medium was separated from testicular tissue by centrifugation and stored at −20°C until steroid hormone assay. The procedure of radioimmunoassay of testosterone was described previously (Yu and Shen, 1989).

**Ovarian incubations and estradiol-17β radioimmunoassay**

After decapitation, ovaries were removed and transferred to large petri dish that contained HBSS (pH 7.40). Follicles were removed from ovarian pieces with forceps and filtered through stainless mesh (Diameter of 1.68 mm. Mesh No. 12) to remove connective fibers and unseparated blocks of follicles. The isolated follicles were washed twice with HBSS (pH 7.40) and then suspended in incubation medium. A scoop-spetula was used to transfer follicles to incubation vial (each incubation vial contained approximately 30 follicles) for subsequent incubation. The other incubation conditions were identical to those for testicular incubations, as described above. The procedure of radioimmunoassay of eatradiol-17β was described previously (Yu and Shen, 1989).

**Cyclic AMP accumulation of common carp ovary**

The effects of various piscine GTHs and tetrapod LHs and FSHs on cyclic AMP formation by common carp ovary were investigated. The incubation conditions for ovarian cyclic AMP formation were identical to those for estradiol-17β production, except that the incubation period was 1 h and 1 mM of MIX was used. Time course patterns of cyclic AMP formations of common carp ovary were also studied. At the end of incubation, the media were separated from the follicles and were transferred to glass tube which were then capped and heated in boiling water for 10 min. The amounts of cyclic AMP in the incubation medium were determined by competitive protein-binding method (Brown *et al.*, 1971; Tsang *et al.*, 1972). The cyclic AMP binding protein was prepared from fresh bovine adrenal cortex by our laboratory based on Gillman's preparation method (Gillman, 1970).

**Statistical analysis**

The statistical calculations of regression, linearity and potency

estimated between different preparations of the hormone were performed by computer program (STATGRAPHICS) according to the statistical equations and the methods described by Finney (1964).

## RESULTS

### Testosterone formation of teleost testes in response to gonadotropins

*Time course pattern of testosterone formation by common carp testis*

The time course pattern of testosterone formation by common carp testis is shown in Fig. 1. As indicated, the amounts of testosterone in the incubation medium were increased linearly during the first 3-4 h of incubation, approached plateau afterwards, and remained constantly high during the following 20 h of incubation, when the testes were stimulated with 50 ng of 37K-scGTH, 10 ng of duck LH, or 10 ng of chicken LH. Testosterone formations were gradually increasing at much lower rate during the 24 h-incubation period for the testis of

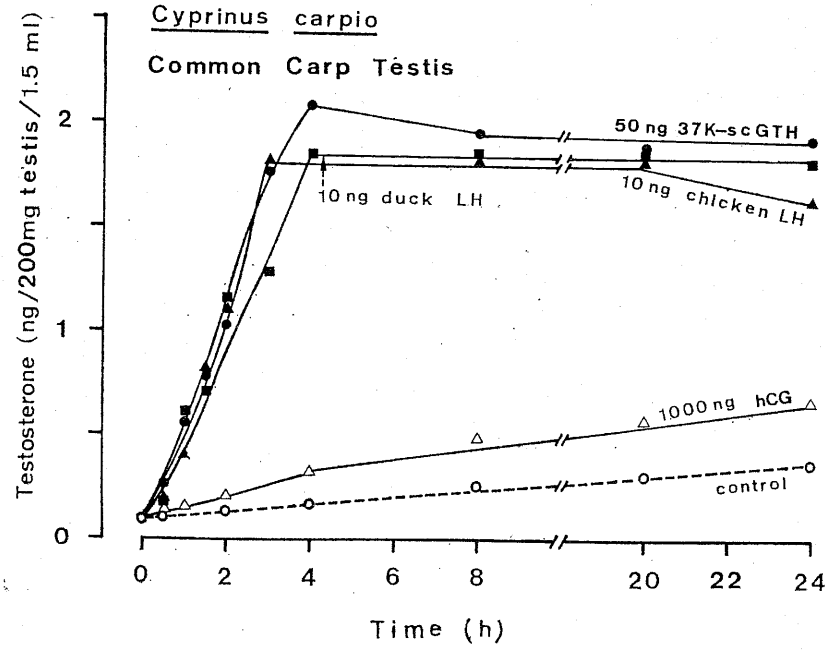


Fig. 1. Time course patterns of testosterone formation of common carp testis (*Cyprinus carpio*) in response to gonadotropins. Testicular tissues (200 mg/vial) were incubated with or without 37K-scGTH, chicken LH and hCG at 25°C for various time intervals during a 24 h-incubation period. The data are expressed as mean of duplicate assays from a single representative incubation experiment.

controls (testes without GTH stimulation) and testes stimulated with 1,000 ng of hCG. Based on the results observed in the time course pattern of testosterone formation, a 4 h-incubation period was thus chosen for studying the responses of testosterone formation by testes from common carp and other teleost species.

*Common carp* (**Cyprinus carpio**) *testis*

As indicated in Fig. 2, testes from common carp were ,highly responsive to piscine GTHs (grass carp, black silver carp and loach). All FSHs tested were inactive in this assay. Sea turtle LH, bullfrog LH, ovine LH and hCG were neither active even at large doses used. All avian LHs were more active than the tested piscine GTHs. Avian LHs and piscine GTHs produced dose-related testosterone formation curves which, in general, paralleled one another. FSHs from tetrapods were not active in this system.

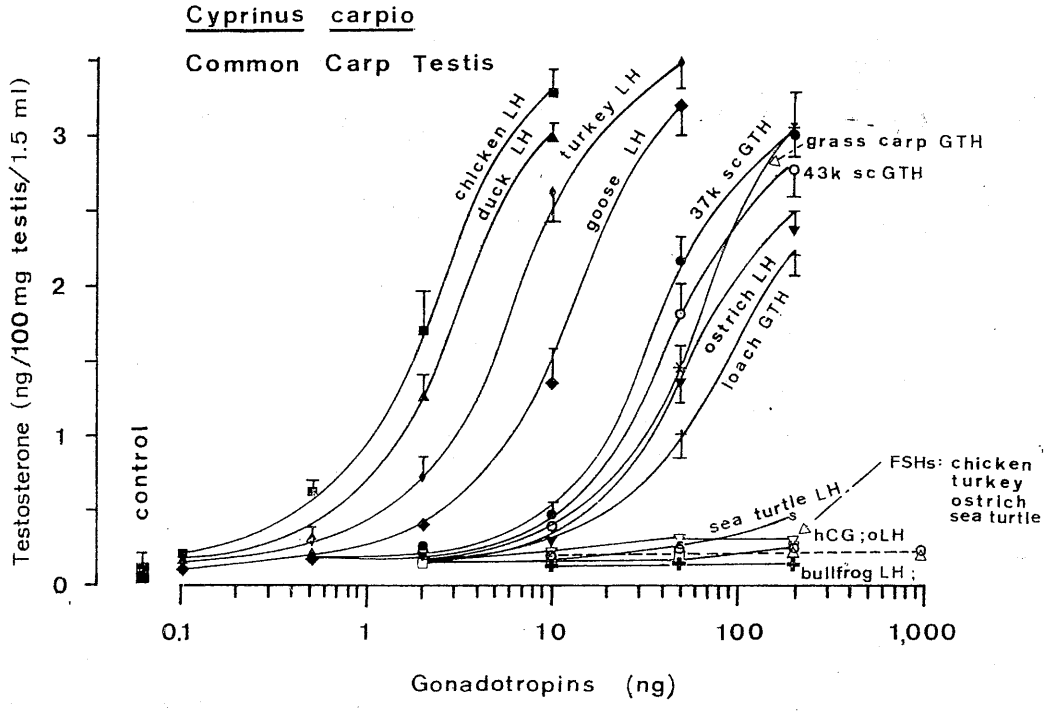


Fig. 2. Testosterone formation of common carp testis in response to piscine GTHs and tetrapod LHs and FSHs. The data are expressed as mean±SD of triplicate assays from a single representative incubation experiment. None of the dose response curves of avian LHs and piscine GTHs significantly nonparallel to each other ($p<0.05$).

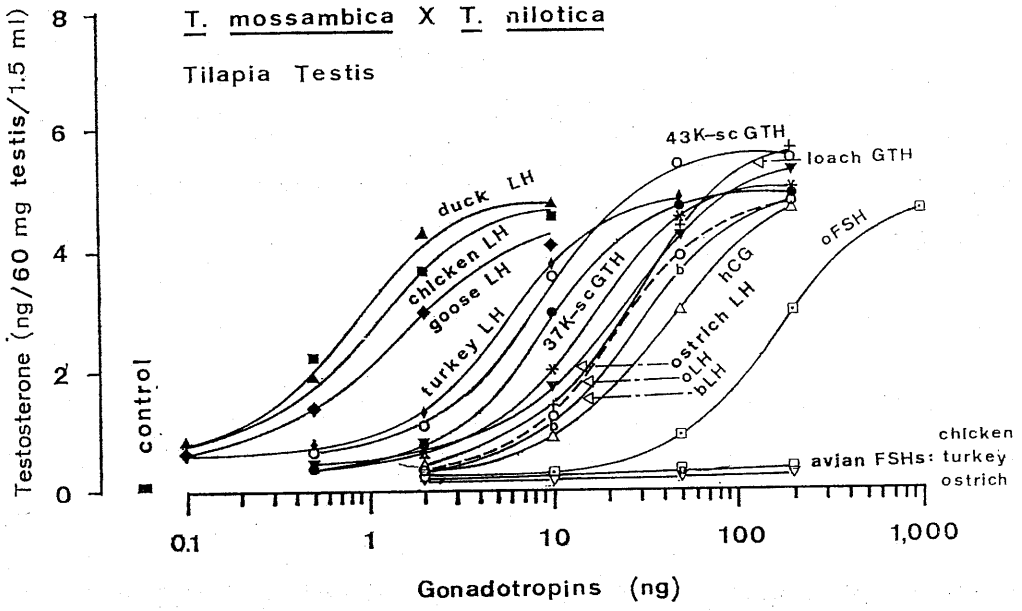


Fig. 3. Testosterone formation of tilapia (*Tilapia mossambica*×*T. nilotica*) testis in response to piscine GTHs and tetrapod LHs and FSHs. The data are expressed as mean of duplicate assays from a single representative incubation experiment. None of the dose-response curves of avian LHs, piscine GTHs, ovine LH and FSH, and hCG significantly nonparallel to each other ($p<0.05$).

*Tilapia* (**Tilapia mossambica**×**T. nilotica**) *testis*

Testosterone formation responses of tilapia testis are shown in Fig. 3. All avian LHs (duck, chicken, goose, and ostrich) were extremely active, although the potencies varied greatly from one to other. LHs from chicken, goose and duck were more active than 43 K-scGTH. Mammalian LHs (ovine and bovine) and hCG were highly active as well. Avian FSHs were inactive in this assay. By contrast, ovine FSH was active, although its potency was only 15% of oLH.

*Swamp eel* (**Fluta alba**) *testis*

Testosterone formation patterns of *Fluta alba* testis in response to gonadotropins are shown in Fig. 4. As indicated, swamp eel testis was very responsive to scGTHs and ovine LH as well as hCG. Avian LHs, however, were less active, in comparison to ovine LH and hGG, in evoking testosterone formation by swamp eel testis. All mammalian and avian FSHs were essentially inactive in this assay system.

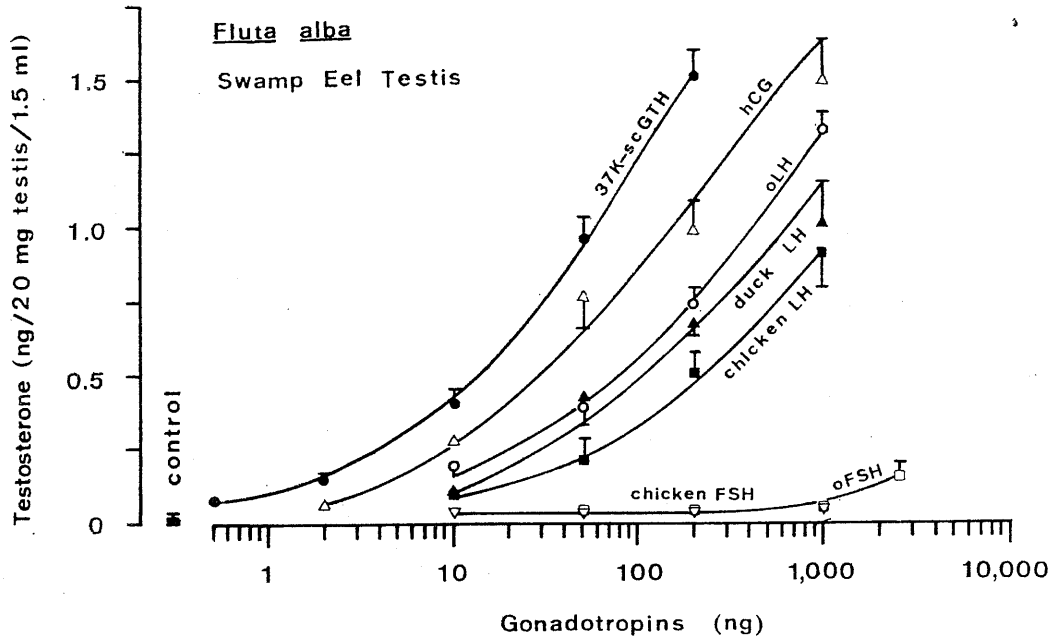


Fig. 4. Testosterone formation of swamp eel (*Fluta alba*) testis in response to piscine GTHs and tetrapod LHs and FSHs. The data are expressed as mean±SD of triplicate assays from a single representative incubation experiment. None of the dose response curves of avian LHs, scGTHs, ovine LH and hCG significantly nonparallel to each other ($p<0.05$).

**Estradiol-17β formation of teleost ovaries in response to gonadotropins**

*Time course patterns of steroids and cAMP formation by common carp ovarian follicles*

The time course patterns of testosterone and estradiol by common carp ovarian follicles are indicated in Figs. 5 and 6. Linear increases of testosterone formation were observed during the first 4 h of incubation of common carp ovarian follicles upon stimulations with 10 ng of duck LH, 10 ng of chicken LH and 100 ng of 37 K-scGTH. The increases of testosterone were slowed down from 4 h to 8 h of incubation, and the amounts of testosterone were markedly decreased at 20 h and 24 h of incubation (Fig. 5). In contrast, the amounts of estradiol-17β in the incubation medium were increased linearly during the first 4 h of incubation, but were increased less rapidly from 4 h to 20 h of incubation and then were slightly decreased during the next 4 h incubation. The amount of estradiol-17β in incubation medium at 4 h incubation period was already about 50% of that of the peaked estradiol-17β at 20 h of incubation.

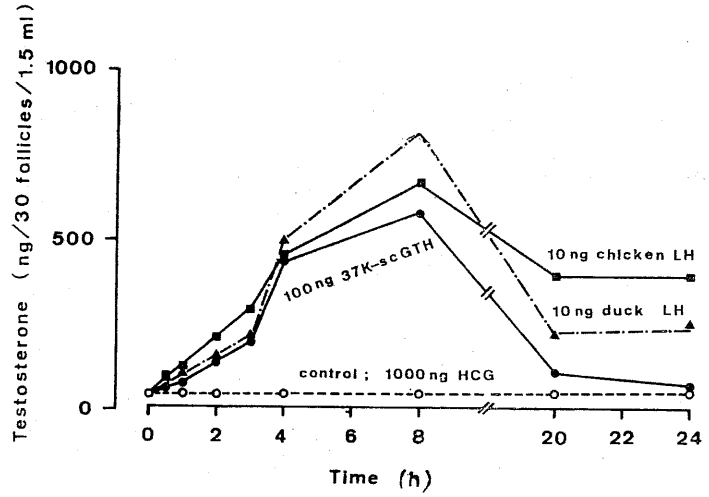


Fig. 5. Time course patterns of testosterone formation of common carp ovary (*Cyprinus carpio*) in response to gonadotropins. Ovarian follicles (40 follicles/vial) were incubated with or without scGTH, chicken LH, duck LH, and hCG at 25°C for various time intervals during a 24 h-incubation period. The data are expressed as mean of triplicate assays from a single representative incubation experiment. The corresponding patterns of estradiol-17$\beta$ formation are indicated in Fig. 6.

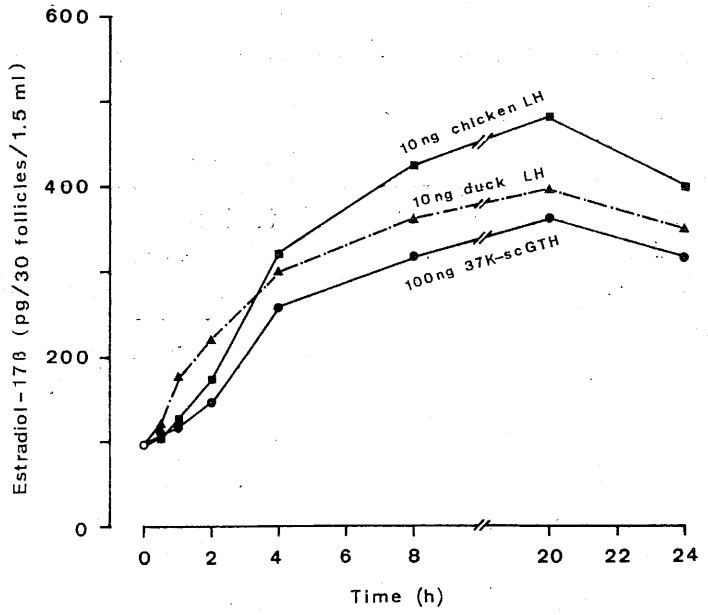


Fig. 6. Time course patterns of estradiol-17$\beta$ formation of common carp ovary (*Cyprinus carpio*) in response to gonadotropins. Ovarian follicles (40 follicles/vial) were incubated with or without scGTH, chicken LH, duck LH, and hCG at 25°C for various time intervals during a 24 h-incubation period. The data are expressed as mean of triplicate assays from a single representative incubation experiment. The corresponding patterns of testosterone formation are indicated in Fig. 5.

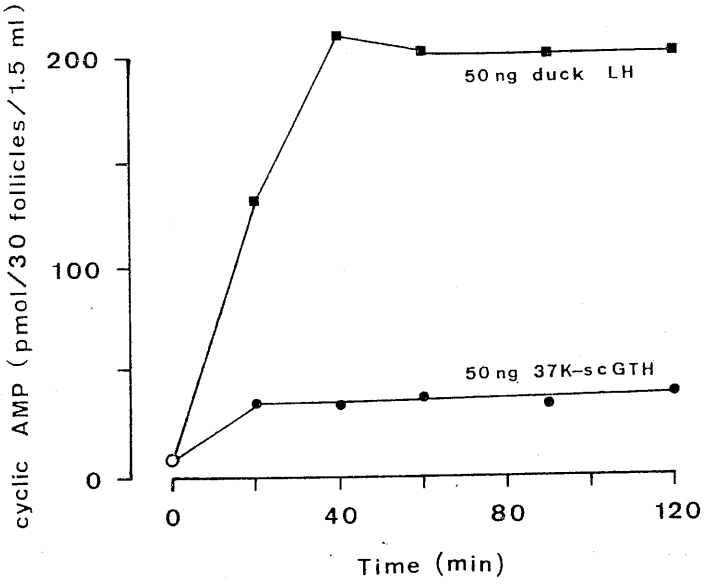


Fig. 7. Time course patterns of cyclic AMP accumulation of common carp ovary (*Cyprinus carpio*) in response to scGTH and duck LH. Ovarian follicles (40 follicles/vial) were incubated with or without gonadotropins at 25°C for various time intervals during a 2 h-incubation period. The data are expressed as mean of duplicate assays from a single representative incubation experiment.

Progesterone levels were virtually undetectable during the 24 h-incubation period (The data are not shown). We chose 4 h-incubation period in the present study for estradiol-17$\beta$ formation of ovarian follicles from common carp, silver carp and rainbow trout. We also conducted a study of the time course of cyclic AMP formation by common carp ovary. As shown in Fig. 7, cAMP was linearly increased, upon stimulation with 50 ng of duck LH, during the first 40 min of incubation and remained in plateau afterwards during a 2 h-incubation period; while cAMP reached the plateau at 20 min of incubation, upon stimulation with 50 ng of 37 K-scGTH. The cAMP accumulation of the common carp ovary as evoked by duck LH was approximately 5-fold greater than that evoked by 37 K-scGTH.

*Common carp* (**Cyprinus carpio**) *ovary*

Estradiol-17$\beta$ formation responses of common carp ovarian follicles are shown in Fig. 8. The ovarian follicles were highly responsive to piscine GTHs and avian LHs; dose-related and parallel estradiol formation

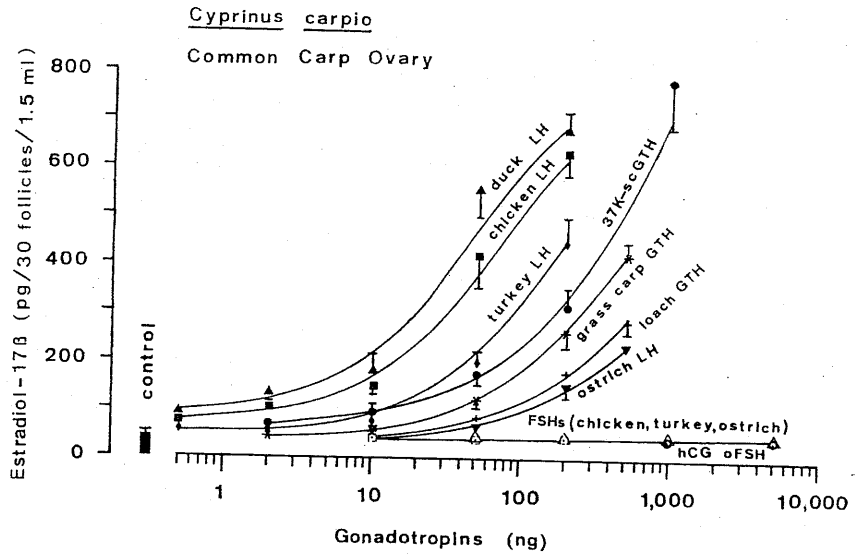


Fig. 8. Estradiol-17$\beta$ formation of common carp ovary in response to piscine GTHs and tetrapod LHs and FSHs. The data are expressed as mean±SD of triplicate assays from a single representative incubation experiment. None of the dose response curves of avian LHs and piscine GTHs significantly nonparallel to each other ($p<0.05$).

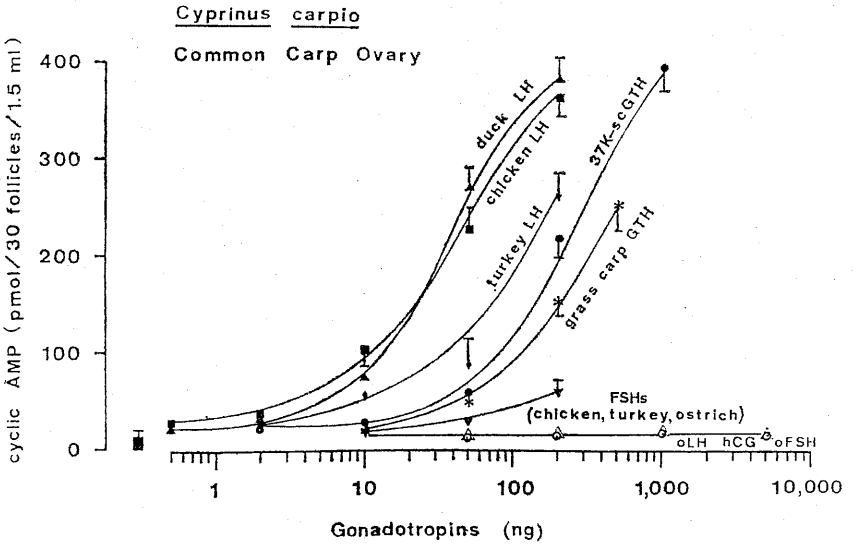


Fig. 9. Cyclic AMP accumulation in the incubation medium of common carp ovary in response to piscine GTHs and tetrapod LHs and FSHs. Incubation was carried out for 1 h. The data are expressed as mean±SD of triplicate assays from a single representative incubation experiment. None of the dose response curves of avian LHs and piscine GTHs significantly nonparallel to each other ($p<0.05$).

Table 3
Relative potency of gonadotropins in stimulation of steroid hormone and cyclic AMP formations by teleosts gonads *in vitro*[a]

| Source of Hormones | | Common carp | | | Silver carp | Tilapia | Swamp eel | Rainbow trout |
|---|---|---|---|---|---|---|---|---|
| Vertebrate Class | Species and Type | Male ($T$)[b] | Female ($E_2$)[b] | (cAMP) | Female ($E_2$)[b] | Male ($T$)[b] | Male ($T$)[b] | Female ($E_2$)[b] |
| Piscine | 37K-scGTH | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 43K-scGTH | 0.86 | 0.75 | ND[c] | ND | 1.25 | ND | 2.0 |
| | gcGTH | 0.75 | 0.67 | 0.67 | ND | 0.63 | ND | 1.5 |
| | loach GTH | 0.33 | 0.33 | ND | ND | 0.40 | ND | ND |
| Mammalian | ovine LH | 0.002 | 0.004 | 0.004 | 0.01 | 0.33 | 0.2 | 0.002 |
| | bovine LH | 0.001 | ND | ND | ND | 0.01 | ND | ND |
| | hCG | 0.001 | 0.004 | 0.004 | 0.01 | 0.2 | 0.5 | 0.002 |
| | ovine FSH | 0.001 | 0.004 | 0.004 | 0.01 | 0.05 | 0.002 | 0.002 |
| Avian | duck LH | 14.3 | 8.0 | 5.7 | 2.0 | 10.0 | 0.1 | ND |
| | chicken LH | 16.7 | 5.7 | 5.7 | 1.9 | 8.33 | 0.05 | ND |
| | chicken FSH | 0.01 | 0.02 | 0.02 | 0.002 | 0.01 | 0.002 | ND |
| | turkey LH | 6.7 | 2.0 | 1.82 | ND | 1.67 | ND | ND |
| | turkey FSH | 0.01 | 0.02 | 0.02 | ND | 0.01 | ND | ND |
| | ostrich LH | 0.06 | 0.25 | 0.20 | ND | 0.50 | ND | ND |
| | ostrich FSH | 0.01 | 0.02 | 0.02 | ND | 0.01 | ND | ND |
| | goose LH | 3.0 | ND | ND | ND | 0.50 | ND | ND |
| Reptilian | sea turtle LH | 0.005 | 0.002 | 0.002 | 0.01 | 0.01 | ND | 0.01 |
| | sea turtle FSH | 0.005 | 0.002 | 0.002 | 0.01 | 0.01 | ND | 0.01 |
| Amphibian | bullfrog LH | 0.005 | 0.002 | 0.002 | 0.01 | 0.01 | ND | 0.01 |
| | bullfrog FSH | 0.005 | 0.002 | 0.002 | 0.01 | 0.01 | ND | 0.01 |

a: The potency estimation between preparations of the hormones was described in Materials and Methods. Relative potencies of all hormones were obtained in relative to 37K-scGTH which was treated as one. The data are expressed as mean of various incubation experiments as indicated in Table 2.
b: T and $E_2$ denote testosterone and estradiol-17$\beta$, respectively.
c: ND: Not detected.

curves were produced by these gonadotropins. They were not responsive to FSHs from birds, bullfrog and sea turtle. Ovine LH and hCG were neither active in this system.

The patterns of cyclic AMP content in incubation medium of common carp ovarian follicles in response to GTHs, are shown in Fig. 9. Dose-related and parallel cyclic AMP formation curves were produced by piscine GTHs and avian LHs. Whereas ovine LH and hCG were inactive in evoking cAMP formations. Relative potencies of responses of testis and ovary of common carp to tetrapod LHs and FSHs, and piscine GTHs are indicated in Table 3.

*Silver carp* (**Aristichythys nobilis**) *ovary*

As shown in Fig. 10, estradiol-17$\beta$ formation responses to tetrapod LHs and FSHs, and piscine GTHs were qualitatively similar to those of common carp ovary. Avian LHs were highly active, while mammalian (ovine) LH and hCG, as well as sea turtle LH and FSH, and bullfrog LH and FSH were inactive in this assay system.

*Rainbow trout* (**Salmo gairdneri**) *ovary*

Rainbow trout ovary was very

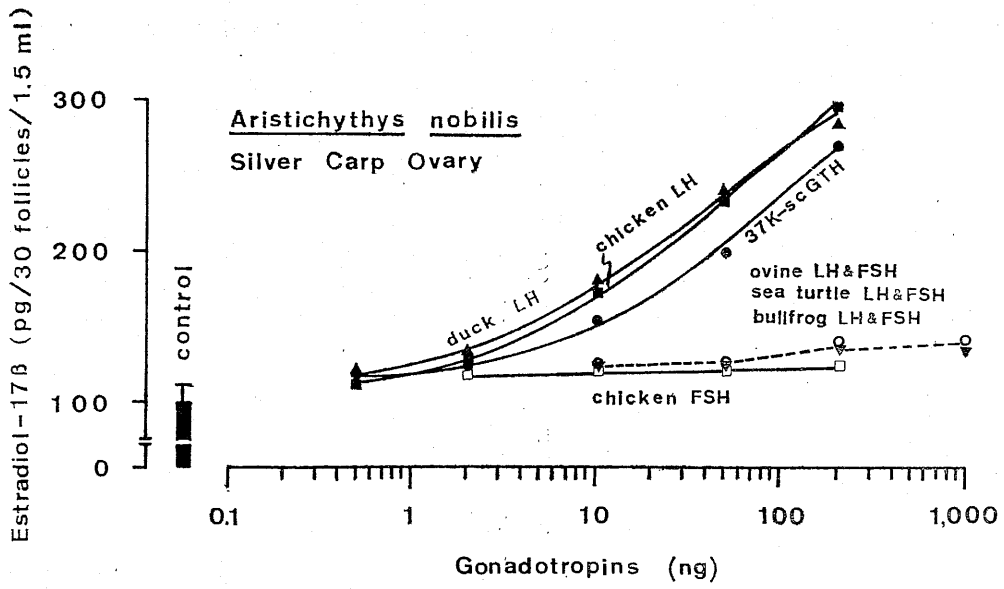


Fig. 10. Estradiol-17$\beta$ formation of silver carp (*Aristichythys nobilis*) ovary in response to piscine GTHs and tetrapod LHs and FSHs. The data are expressed as mean±SD of triplicate assays from a single representative incubation experiment. None of the dose response curves of avian LHs and piscine GTHs significantly nonparallel to each other ($p<0.05$).

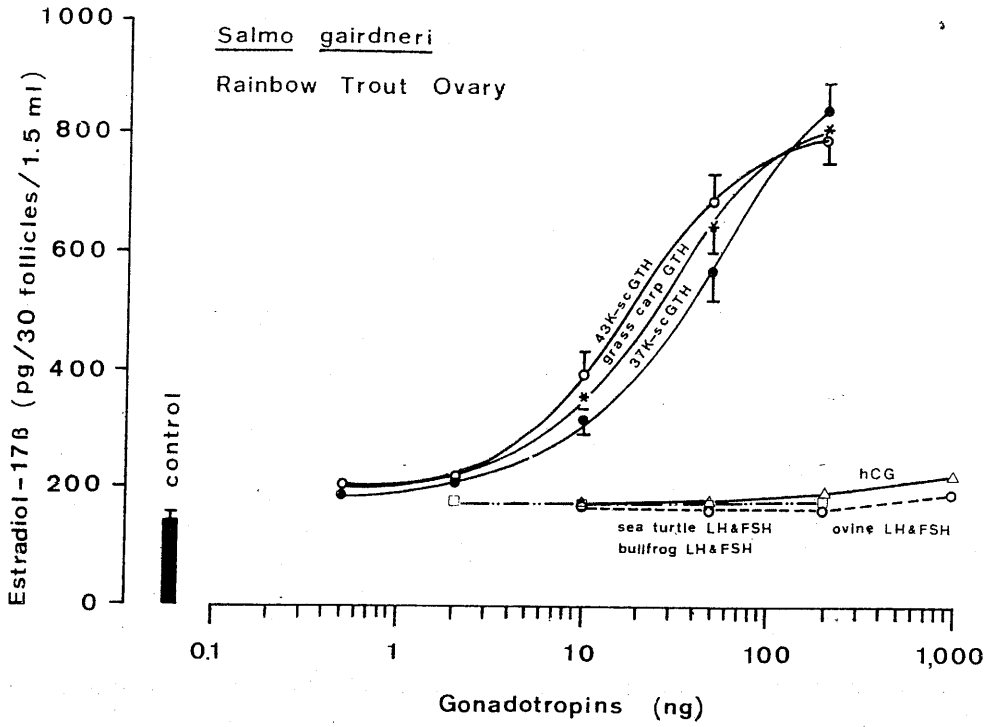


Fig. 11. Estradiol-17$\beta$ formation of rainbow trout (*Salmo gairdneri*) ovary in response to piscine GTHs and tetrapod LHs and FSHs. The data are expressed as mean±SD of triplicate assays from a single representative incubation experiment. None of the dose response curves of avian LHs and piscine GTHs significantly nonparallel to each other ($p<0.05$).

responsive to black silver carp GTH (37 K and 43 K) and grass carp GTH (Fig. 11) in stimulating estradiol-17$\beta$ formation. Ovine LH and hCG were neither active at doses up to 1,000 ng/vial.

## DISCUSSION

### Gonadal response characteristics

The present study reveal that the sensitivity and the amount of steroid formation by teleost gonads from a single species upon stimulation with gonadotropins depend largely on the stages of gonadal maturation; no evidence is observed regarding seasonal changes of gonadotropin specificity or species specificity among the fish studied here. These observations are comparable with those reported by others on different species of teleosts (Bona-Gallo and Licht, 1981, 1983; Yu and Lin, 1985). All these findings suggest that the number and/or binding activity of GTH receptor in teleost gonad alter during various stages of reproduction,

but the type of GTH receptor appears to be uniform without changes along with seasonal cycle or withe gonadal maturation.

We found that more mature testes (particularly near spermiation) and preovulatory ovaries were much less responsive to gonadotropins in steroid formation, and that the sensitivity and the magnitude of response of more mature gonads (fold increase of steroid formation from the control basal value to the maximal) were subsequently lower than those produced by testes from developing stage and vitellogenic, but not the preovulatory, ovaries. The amount of steroids formed by gonads in response to gonadotropins also varied between species; however, testes and/or ovaries from all five species of teleosts, examined in the present study, were highly responsive to silver carp GTH which was used as a reference hormone.

Different categories of androgen and estrogens are formed by gonads from different species of teleosts (Campbell *et al.*, 1976; Dodd *et al.*, 1978; Idler and Ng, 1979; Manning and Kime, 1984; Sakai *et al.*, 1989). And different precursor (intermediate) steroids are secreted to the medium during incubation of the teleost gonads (Kime and Hyder, 1983; Manning and Kime, 1984; Sakai *et al.*, 1989). In our study, we illustrated that testosterone in the medium can also be used as an index of steroid formation by ovary from common carp since there was a parallelism in the content in incubated medium between the two steroids during certain period of incubation. In several teleost species, 11-ketotestosterone (11-KT) is the major androgen in the plasma of the male in comparison to testosterone (Campbell *et al.*, 1976; Dodd *et al.*, 1978; Idler and Ng, 1979). Evidences were indicated that parallel changes of 11-KT and testosterone occur during sexual maturation (Dodd *et al.*, 1978), or seasonal cycles (Campbell *et al.*, 1976). However, changes in the ratio of the two steroids were observed for *in vivo* responses to fish gonadotropin (Idler and Ng, 1979).

Under the *in vitro* incubation conditions, we have shown that increase of testosterone in the incubation medium is an appropriate index of enhanced androgen formation by testes of various teleost species examined in the present study and in previous investigations (Bona-Gallo and Licht, 1981; Yu and Lin, 1985, 1986; Yu and Shen, 1989). Nevertheless, differences in the relative importance of testosterone

between species or stage of testis maturation are likely to account for the variation observed in the sensitivity or the amount of testosterone produced by the teleosts examined in this study.

Thus, the results regarding the responses of teleosts from five species (representing four family of four orders) to hormones derived from representatives of teleosts and all tetrapod classes, provide a basis for evaluation of two properties of the hormone receptor interaction underlying the regulation of gonadal steroid formation and secretion. The relative responsiveness of the gonad (testis or ovary) to the two types of gonadotropins (LH and FSH) is designated as gonadotropin specificity, and the variation in activity of gonadotropins derived from different species is designated as species specificity. The designations of the two properties of the hormone-receptor interaction in the present study are identical to those employed by Bona-Gallo and Licht (1981, 1983) conducting similar studies on different teleost species.

**Gonadotropin specificity**

Results from the present study indicated that testicular testosterone formation in teleost testis exhibits a great diversity in gonadotropin specificity. Testes from each of the three teleost orders represented in this study show a distinct specificity to avian LHs; while avian FSHs are inactive; tilapia (Order Perciformes) and *Fluta alba* (Order Synbranchiformes) exhibit high specificity to mammalian (ovine) LH and placental gonadotropin (hCG), while *Cyprinus carpio* (Order Cypriniformes) is unresponsive to ovine LH and hCG. *Cyprinus carpio* testis is not responsive to LHs and FSHs from reptilian (sea turtle) and amphibian (bullfrog). Mammalian (ovine) FSH is inactive in *Cyprinus carpio*, slightly active in *Fluta alba*, but rather active in tilapia (*Tilapia mossambica* × *T. nilotica*) although being less potent as compared to ovine LH.

Bona-Gallo and Licht (1981) examined the gonadotropin specificity of four species of teleost from three different families. They demonstrated that testes of *Gilichthys mirabilis* (Family Gobiidae) are highly specific for all tetrapod LHs (FSHs are inactive) with mammalian and amphibian LHs being more potent than reptilian and avian LHs. In contrast, testes of *Cichlasoma citrinellum* and *Sarotherodon mossambicus* (Family Cichlidae) lack gonadotropin specificcity (most preparations of

FSH and LH are about equi-potent), and in *C. citrinellum* all species of tetrapod LHs and FSHs are active. In *Salmo gairdneri* (Family Salmonidae) there is specificity of amphibian (bullfrog) LH, but not for mammalian (ovine) LH. We also demonstrated the testes of crucian carp (*Carassius auratus* × *C. cuveri*) and catfish (*Clarias macrocephalus*) are more responsive to ovine LH than ovine FSH in androgen formation (Yu and Lin, 1985).

Since the species of teleosts studied differed entirely and the gonadotropins assayed differed partially between our present study and that of Bona-Gallo and Licht (1981). It thus facilitates a comparison of gonadotropin specificity in teleost for illustrating the variability of between species, families or orders. Although the gonadotropin specificity of androgen formation by testes of the teleost observed in the two studies differed rather greatly, the teleost testes, in general, are more responsive to tetrapod LHs than FSHs of the same species. The findings from this study together with previous observations (Bona-Gallo and Licht, 1981; Yu and Lin, 1985) clearly indicate that testes of teleost have high degree of interspecific variability in response to tetrapod LH and FSH in androgen formation.

In mammals and birds, distinct LH-specificity exists in testicular androgen formation (Licht *et al.*, 1977; Pierce and Parson, 1981; Yu and Wang, 1987); in reptile, certain species show a general lack of gonadotropin specificity, while others exhibit high LH specificity (Licht and Pearson, 1969; Tsui and Licht, 1977; Licht and Papkoff, 1985; Yu *et al.*, 1991); and most of amphibia examined show a high LH specificity (Licht and Papkoff, 1974; Muller, 1977; Muller and Licht, 1979; Yu *et al.*, 1991). The present study together with other investigations (Bona-Gallo and Licht, 1981, 1983) demonstrate that either distinct or high LH-specificity exists in teleost, depending on species, The findings from these studies on gonadotropin specificity in represented species of various vertebrate classes suggest that the existence of LH-specific receptor in testes already occurred before the differentiation of pituitary gonadotropin to LH and FSH that appeared firstly in amphibia.

Gonadotropin specificity of estradiol-17$\beta$ formation responses of silver carp ovary are very much similar to that observed for common carp ovary. It has been shown that

ovine LH is much more active than FSH in stimulating estradiol-17$\beta$ formation by ovarian follicles from amago salmon (*Oncorhynchus rhodurus*) (Kagawa *et al.*, 1982). In studying of the gonadotropin specificity of ovarian estrogen formation by teleost, Bona-Gallo and Licht (1983) demonstrated that *Gillichthys mirabilis* (one species of gobiid), and two cichlids, (*Cichlasoma citrinellum* and *Sarotherodon mossambicus*) respond to both types of tetrapod gonadotropins (LHs and FSHs); the gobiid ovary showed highest LH specificity while in the two cichlids the potencies of FSHs ranging from 11 to 100% of the responsive LHs. Results from the present study and together with findings by others (Kagawa *et al.*, 1982; Bona-Gallo and Licht, 1983) clearly indicate that great interspecific variability exists in teleost ovarian response to tetrapod LH and FSH. Nevertheless, in general, LHs are more active than FSHs in stimulating estradiol-17$\beta$ formation by teleost ovaries.

**Species specificity**

Studies on species specificity of pituitary gonadotropic hormone could be dated back to more than fifty years ago. In 1929, Houssay found that pituitary implants from different vertebrate classes could not duplicate the phenomenon of ovulation induction as it was produced in the toad by homoplastic implants (Houssay *et al.*, 1929). He was probably the first one to advance the hypothesis of species specificity of gonadotropic hormone. A review on the species specificity of gonadotropic factors in vertebrates was published by Creaser and Gorbman in 1939. Investigators implanted fresh pituitaries, or injected pituitary extracts prepared from various vertebrate species into fish, amphibia, reptilia and birds; they then examined the subsequent effects on the stimulation of gonadal growth or ovulation in the recipient animals. It was stated by Creaser and Gorbman (1939), "... it is concluded that a qualitative species variation exists in gonadotropic hormones. This variation may be great enough between widely separated donor and recipient species to lead to an apparent ineffectiveness of the hormone." Today, we are investigating the similar question, but with defined hormonal molecules and specified functional parameters (*in vitro* steroid or cyclic AMP formation bioassays) to explore the species specificity at the level of hormone-receptor interactions.

Comparison of the relative

potencies of gonadotropins from different tetrapods and teleosts in fish testis and ovary bioassays in the present study revealed that: 1) tremendous variability exists in the potencies of tetrapod LHs when assayed in either testis or ovary from a single species of teleost; and 2) a great degree of interspecific difference exists in either testis or ovary among teleosts in response to tetrapod LHs. Consequently, no clear correlation with the phylogenic relationship is observed with respect to evolution of LH molecules among tetrapod vertebrates studied. Among the five species reported in this study and three other teleost species (our unpublished data), we found all of them except *Salmo gairdneri*, are highly responsive to avian LHs, and four teleost species (testes from tilapia, *Tilapia mossambica* × *T. nilotica;* swamp eel, *Fluta alba*; crucian carp, *Carassius aurats*×*C. cuveri*; and catfish, *Claris macrocephalus*) are responsive to mammalian (ovine) LH, although being less potent, in general, than avian LHs.

The existence of specific binding sites for GTH was demonstrated in gonads from several teleost species (Aida and Ishii, 1981; Van der Kraack and Donaldson, 1982; Salmon *et al.*, 1985; Bieniarz and Kime, 1986). Evidences indicate that teleost GTH, like mammalian and other tetrapod LH and FSH, act *via* binding to gonadal adenyl cyclase-coupled receptor (Fontaine, 1980). Formation of cyclic AMP by teleost gonads has been used for measurement of biological activity of teleost gonadotropins (Fontaine *et al.*, 1972; Salmon, *et al.*, 1985; Ando and Ishii, 1988). The present study has shown the parallelism in the dose response curves between cyclic AMP and estradiol-17$\beta$ of the common carp ovaries responding to various piscine GTHs and avian LHs. These observations indicate that both piscine GTHs and avian LHs bind to the same GTH-receptors of the ovaries. The greater formation of cyclic AMP observed for avian LHs would reflect greater activation of adenyl cyclase, which presumably resulting from greater binding of the hormones to ovarian receptors. Further studies on the hormone-receptor interactions are required to explain the greater activities of avian LHs in stimulation of the gonadal steroidogenesis in most of the teleosts examined in the present study.

Bona-Gallo and Licht (1981, 1983) showed that considerable species specificity exists in testes and ovaries of teleosts, in response to stimulation

of tetrapod GTHs or piscine GTH. Testes of *Gillichthys mirabilis* show a certain degree of species specificity; mammalian and amphibian LHs are much more potent than reptilian and avian LHs; in contrast, testes of *Cichlasoma citrinellum* are more responsive to reptilian (sea turtle) LH. In general, the species specificity of the ovaries parallel that observed in the testes of the same teleost species. They thus indicated that the lack of predictability in the pattern of species specificity from the standpoint of the recipient or donor illustrates further the complex patterns of radiant and convergent evolution that may have occurred in glycoprotein hormones (Licht and Papkoff, 1974; Bona-Gallo and Licht, 1981). The findings that the species specificity of gonadal steroidogenic response in teleost to tetrapod and piscine gonadotropins, as observed in this study and in other studies (Bona-Gallo and Licht, 1981, 1983; Yu and Lin, 1985), illustrates the variability of the structure of their gonadal receptors. The variability and unpredictability of such species specificity observed by these studies, suggest that the results based on one species of fish should not be extrapolated to other species, and particularly true for the species from different families and/or orders.

The potencies of GTHs derived from pituitaries of silver carp, grass carp, common carp and loach, assayed on both testis and ovary of common carp, are very similar. Such homology in biological potencies of these hormones is in consistency with the results observed in the homology of their amino acid sequences (Chang *et al.*, 1990; Lo *et al.*, 1991). We have previously shown that salmon GTH, in comparison with silver carp GTH and common carp GTH, is relatively less active in stimulating androgen formation *in vitro* by common carp testis (Yu and Lin, 1986). Again, such findings on biological potencies are also consistent with the results that smaller extent of homology existing in amino acid sequence between salmon GTH and GTHs from common carp and silver carp (Chang *et al.*, 1990; Lo *et al.*, 1991). The findings that the resemblance of the potencies among these teleost GTHs, assayed on a teleost system, thus virtually reflect the resemblance of their structure. The present study also demonstrated that the gonad from all five teleost species examined are highly responsive to stimulation with silver carp GTH, implicating that the phylogenic resemblance during

evolutionary process exists in both GTH molecules and gonadal receptors among most of the species, if not all, within the Class Osteichthyes.

**Sexual difference of gonadal response**

The results of the present study revealed that the response of estradiol-17$\beta$ formation by common carp ovary to piscine GTHs and tetrapod LHs and FSHs are similar to the response of testosterone formation by common carp testis, in terms of gonadotropin specificity and relative potencies of the gonadotropins. One noticeable sexual difference in gonadal responses to gonadotropin is that the species specificity of gonadotropine evoking ovarian estradiol-17$\beta$ formation by common carp appears to be more homogenous than that of testicular testosterone formation in the male. These observations are consistent with the findings on different species of teleosts (*Gillichthys mirabilis*, *Cichlasoma citrinellum* and *Sarotherodon mossambicus*) reported by Bona-Gallo and Licht (1983). They showed that the specificity of ovarian estradiol-17$\beta$ formation patterns, responding to gonadotropins, parallel to those observed for testosterone formation in the males of the same fish, but the differential actions of the tetrapod hormones (both species specificity and hormonal specificity) are more exaggerated for the testes. All these findings indicate that there appears to be no sex difference in GTH-receptor between testis and ovary with respect to the active site of the gonadal receptors for interaction with gonadotropin.

**Potential application in aquaculture**

The present study revealed that avian LHs (chicken, turkey, ostrich and duck) are highly active in stimulating testosterone formation by testes from common carp, tilapia and silver carp, and estradiol-17$\beta$ formation by ovarian follicles from common carp and silver carp. The potencies of certain avian LHs are even one order greater than those of the piscine GTHs. The findings from the present study, thus, provide a basis for further investigation of potential application of avian LHs in aquaculture with respect to enhancement of steroidogenesis which relates to spermatogenesis, vitellogenesis and other processes of reproduction. In fact, we have shown that duck LH and duck pituitary gland extract are potent ovulation stimulants in loach (Yu and Liu, 1991). Further studies in the potential applications of avian

LHs in fish aquaculture are strongly encouraged.

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