Bull. Inst. Zool., Academia Sinica **32**(3): 194-203 (1993)

Establishment of an *In Vitro* Bioassay for Measuring Duck Serum Luteinizing Hormone Levels: Rooster Testicular Testosterone Formation System[1]

San-Tai Shen and John Yuh-Lin Yu[2]

Endocrinology Laboratory, Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan 115, R.O.C.

(Accepted February 22, 1993)

San-Tai Shen and John Yuh-Lin Yu (1993) Establishment of an *In vitro* bioassay for measuring duck serum luteinizing hormone levels: rooster testicular testosterone formation system. *Bull. Inst. Zool., Academia Sinica* **32**(3): 194-203. The purpose of this study was to establish a simplified rooster dispersed testicular cell testosterone formation system for an *in vitro* bioassay of purified luteinizing hormones (LHs) and circulating LH of ducks. Dispersed testicular cells were prepared from 3- to 4-month-old roosters. Five grams of decapsulated testes were dispersed in 50 ml Medium 199, first with a dropper, then with a 25-ml glass syringe, until a homogeneous suspension was obtained. Following preincubation at 37°C for 0.5 hr, dispersed testicular cells (5×10^6 interstitial cells/vial) were incubated with various doses of LHs, pituitary extract, or serum in Medium 199 with 0.125 mM MIX (pH 7.40) at 37°C for 4 hr in a Dubnoff incubator shaking at 100 cycles/min under continuous aeration of 95% O_2 – 5% CO_2. Testosterone levels in the incubated medium were then measured by radioimmunoassay.

Parallel dose-related testosterone formation curves were produced with mammalian LHs, avian LHs, and piscine gonadotropin (GTH); other pituitary hormones (follicle stimulating hormone, thyroid stimulating hormone, growth hormone, and prolactin) were ineffective in inducing testosterone formation. The sensitivity of this system was measured at 0.1 ng/vial and 0.2 ng/vial for purified ovine LH (NIADDK-oLH-25) and duck LH (ASIZ-dLH-1), respectively. Duck sera produced dose-related testosterone formation curves parallel to purified duck LH. Elevated LH levels in the blood circulation of ducks following GnRH injection were successfully measured by the present method. This system for the testosterone formation of rooster dispersed testicular cells was therefore found to be suitable for *in vitro* bioassays of purified mammalian and avian LHs, as well as the LH contents of duck pituitary and blood serum.

Key words: *In vitro* LH bioassay, Rooster testicular testosterone formation, Vertebrate LHs, Duck pituitary and serum LHs.

The steroidogenesis of gonadal cells in many vertebrate species has been studied *in vitro* and used to assess the biological potency of gonadotropins (GTHs) from homologous or heterologous species. Examples include rats (Dufau *et al.* 1974 1976), mice (Steiner *et al.* 1980, Yu *et al.* 1981),

1. Paper No. 381 of the Journal Series of the Institute of Zoology, Academia Sinica.
2. To whom reprint requests should be sent.

rabbits (Licht *et al.* 1976), pigs (Bernier *et al.* 1983), chickens (Ax 1978, Glenn *et al.* 1981, Yu and Wang 1987), quail (Jenkins *et al.* 1978, Maung and Follet 1977), and piscine (Yu *et al.* 1991); the testicular androgen formation systems of these animals were used for *in vitro* bioassays of gonadotropins. Of these animals, rat and mouse testicular androgen formation systems have been studied most extensively (Van Damme *et al.* 1974, Dufau *et al.* 1976, Farmer *et al.* 1977, Steiner *et al.* 1980, Yu *et al.* 1984). These systems are not only useful for *in vitro* LH bioassays, but also for analyses of the mechanisms of gonadotropins on testicular steroidogenesis and investigations into hormone-receptor interaction (Ding and Huhtaniemi 1989, Haour and Saez 1977, Ramachandran and Sairam 1975). It has been established that mammalian testicular systems are highly responsive to mammalian LHs, but considerably less responsive to the LHs of avian and other lower vertebrate classes (Licht *et al.* 1977, Yu and Wang 1987).

Our laboratory has established both mammalian (mouse and rat) interstitial cell incubation systems and a rooster testicular slice incubation system for *in vitro* bioassays of vertebrate LHs (Yu *et al.* 1981, Yu and Wang 1987). We have observed that the rooster system is more responsive to the LHs of many vertebrate classes than are the mouse and rat systems; thus, the rooster system is suitable for *in vitro* assays of LHs from virtually all vertebrate classes. However, sliced rooster testicular preparation is time-consuming and labor-intensive; in addition, fewer homogeneous preparations are usually obtained. We thus attempted to establish a simplified, mechanically-dispersed rooster testis cell preparation method for testosterone formation *in vitro*, and to further apply this system to quantify circulating duck LH.

MATERIALS AND METHODS

Hormones and chemicals

The ovine LH (NIADDK-oLH-25; 2.3 × NIH-LH-S1), thyroid stimulating hormone (TSH, NIADDK-oTSH-12), growth hormone (GH, NIADDK-oGH-14), and prolactin (PRL, NIADDK-oPRL-18) used in this research were generous gifts from the National Hormone and Pituitary Program, NIADDK, Baltimore, USA. Porcine LH (USDA-pLH-B-1) was provided by the USDA Animal Hormone Program, USA. Chicken LH (CANOMS124326) and follicle stimulating hormone (FSH) (AGCQSQ123445C) were kindly provided by S. Ishii. Duck LH (ASIZ-dLH-1) and grass carp GTH (gcGTH-DEI) were purified in our laboratory (Yu *et al.* 1987, Yu and Shen 1989). Gonadotropin releasing hormone (GnRH), collagenase (Clostridiopeptidase A; EC 3.4.24.3; Type I), 1-methyl-3-isobutyl-xanthine (MIX), bovine serum albumin (BSA, Fraction V), HEPES (N-2-hydroxyethyl piperazine, N-2-ethane sulfonic acid), and penicillin/streptomycin were purchased from the Sigma Chemical Co. St. Louis, USA. Medium 199 (with Hanks' Salts and L-Glutamine) were obtained from GIBCO Laboratories, Chagrin Falls, Ohio, USA. Tritiated testosterone (1, 2, 6, 7, 16, 17-[^{3}H], 168 Ci/m mol) was purchased from New England Nuclear Research Products, Boston.

Animals

Three- to four-month-old Dutch Hybro strain roosters (*Gallus domesticus*) were purchased from a local poultry farm in Taipei. Chickens were raised at a temperature range of 20-25°C with a light schedule of 14L:10D. Food and water were available *ad libitum.*

In Vitro LH bioassay System

Preparation and incubation of mech-

anically dispersed rooster testicular cells. Roosters were sacrificed by decapitation and testes were excised and weighed. Testes (8.7 g – 36 g/pair; GSI 0.31 – 1.0%) were then decapsulated, cut into small pieces (5 g of testes for 100 assays), and placed into 50 ml of preincubation medium (Medium 199 with 25 mM HEPES, 10,000 units penicillin/100 ml, 5 mg streptomycin/100 ml, 0.1% BSA, 0.1% sodium bicarbonate, pH 7.40). Testicular tissue was dispersed with a plastic dropper, then repeatedly drawn into and gently squeezed from a 25 ml glass syringe (minus needle) repeatedly until an homogeneous suspension was obtained. The suspension medium was then filtered through a 2 × 2 mm Nylon mesh to remove tissue pellets, and centrifuged at 100 x *g* for 10 min at 8°C. Cells were then resuspended in 50 ml preincubation medium and preincubated in 100 ml glass beakers at 37°C for 0.5 hr. After preincubation, the cell suspension was centrifuged, and after which cell pellets were resuspended in 50 ml of incubation medium (preincubation medium plus 0.125 mM MIX).

Cells were incubated in 20 ml glass counting vials at 37°C for 4 hr; the vials were vibrated in a Dubnoff metabolic incubator at 100 cycles/min under continuous aeration with 95% O_2 – 5% CO_2. The total volume of each vial was 1.0 ml; in addition, the effects of incubation volumes (2.0 ml and 4.0 ml per vial) on testicular testosterone formation were also studied. Each vial contained 0.5 ml of dispersed cell suspension (50 mg of original tissue, 5 × 10^6 cells) and 0.1 ml of LHs, pituitary extracts, and/or various amounts of serum samples; various volumes of incubation medium were then added to reach a total of 1.0 ml of total incubation volume per vial. After incubation, vials were placed in ice baths for 10 min and 1 ml of 0.01 M PBS (phosphate buffer saline) was added; vials were then stored at −20°C until testosterone radioimmunoassay were performed.

Preparation and incubation of collagenase dispersed rooster testicular cell. Rooster testes were prepared as previously described, except that they were dispersed with collagenase treatment [Clostridiopeptidase A; EC3.4;24,3 Type I] (Yu *et al.* 1981 1984). Briefly, 5 g of testis tissues were incubated with collagenase (10 mg/5 g tissue/10 ml preincubation medium) at 37°C for 15 min and shaken at 100 cycles/min. Testicular cells were washed, filtrated, centrifuged, and incubated as previously described.

Radioimmunoassay of testosterone and calculation of LH activity. Testosterone radioimmunoassay procedures of testosterone were similar to those described by Yu *et al.* (1990). The LH contents of duck pituitary glands and sera were calculated from the regression analyses of testosterone formation curves, using duck LH (ASIZ-dLH-1) as a reference hormone. Mean intra- and inter-assay coefficients of variation of LH bioassays for pooled serum from sexually mature female ducks were 6.5% and 14%, respectively (N = 6). The sensitivity of the bioassay was calculated from the mean baseline of testosterone formation from testes not exposed to the hormone (control) plus two standard deviations.

Bioassay of pituitary LH activity of ducks from two age groups

Pituitary glands were collected from 70- and 250-day-old female Tsaiya ducks (Chinese common duck, *Anas platyrhynchos domesticus*). The glands were immersed in 2 ml of 0.01 M PBS and homogenized by polytron in an ice bath. Serial dilution was achieved by adding 0.01 M PBS; 0.1 ml of diluted pituitary homogenate preparation was used for an LH assay.

Bioassay of duck serum LH: Changes in circulating LH levels of ducks in response

to GnRH stimulation

Female Tsaiya ducks at their sexually developing stage (80-90 days old) were used to study LH release in response to exogenous GnRH stimulation. Ducks were injected with 20 μg of GnRH/kg body weight (in 100 μl saline, subcutaneous injection); control duck were injected with saline only. Blood samples were collected via the tibial vein at -10, 0, 20, 40, 60 and 120 min intervals. Sera was obtained by centrifugation (2000 x *g* for 20 min) and stored at $-20°C$ until LH assays were performed.

Statistical analysis

Statistical calculations of regression, parallelism, and potency estimates between different hormone preparations were performed by the computer program STATGRAPHICA according to statistical equations and methods described by Finney (1964).

RESULTS

Time course patterns of rooster testicular testosterone formation

The time course patterns of testosterone production by rooster dispersed testicular cells (50 mg tissue/ml) following stimulations with ovine LH (NIADDK-oLH-25) are presented in Fig. 1. Control cells (without LH stimulation) produced limited testosterone during the 6 hr incubation period. The testosterone formation of rooster testicular cells stimulated by 2.5 ng of ovine LH increased linearly during the first 4 hr of incubation and maintained a plateau level thereafter. Testosterone amounts produced by rooster testicular cells stimulated by 2.5 ng of ovine LH were more than ten-fold higher than those of controls after 4 hr of incubation. Therefore, a 4 hr incubation period was chosen as the standard for this study.

Effects of collagenase dispersion and volume of incubation medium on testosterone formation of rooster testicular cells

As shown in Fig. 2, rooster testicular cells dispersed by collagenase treatment were unresponsive to ovine LH in stimulating testosterone formation. In contrast, dispersed cells prepared by the mechanical method were highly sensitive in response to ovine LH, producing dose-related levels of testosterone.

The effects of incubation medium volume (1.0 ml, 2.0 ml, and 4.0 ml) on testosterone formation are shown in Fig. 3. Dose-related amounts of testosterone were produced by rooster testicular cells incubated in 1.0 ml, 2.0 ml, and 4.0 ml/vial; the 1.0 ml incubation medium produced the highest amount of

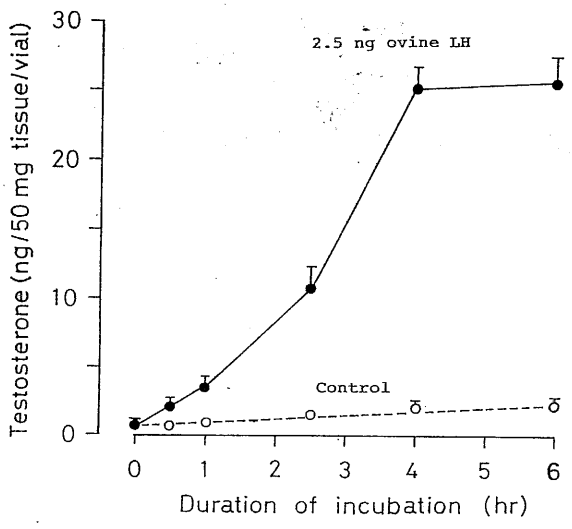

Fig. 1. Time course patterns of testosterone formation by rooster dispersed testicular interstitial cells. Cells (5×10^6 cells/1.0 ml/vial) were incubated with or without 2.5 ng of ovine LH (NIADDK-oLH-25) at 37°C. Data are expressed as mean $\pm$ SEM from a single assay of triplicate incubations.

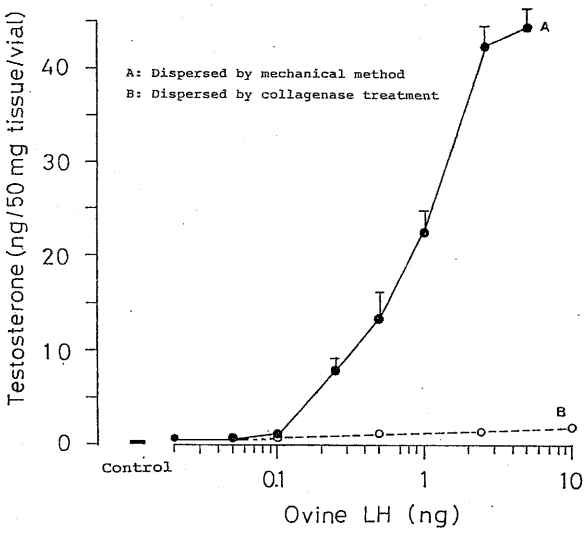

Fig. 2. Comparisons of testosterone formation of rooster testicular cells prepared by mechanical dispersion and collagenase treatment. Data are expressed as mean ± SEM from a single assay of triplicate incubations.

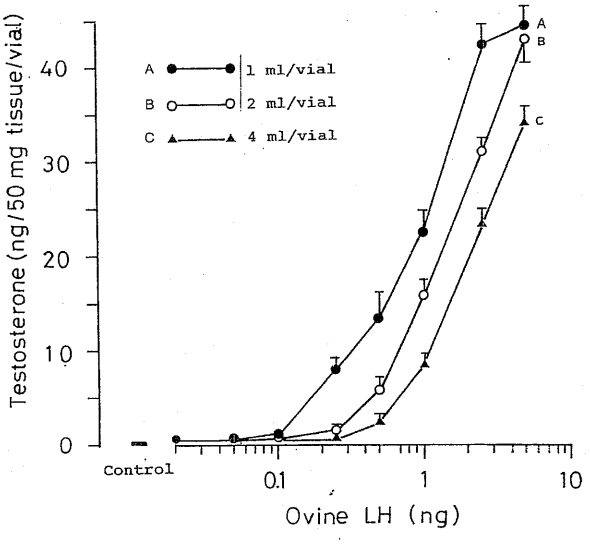

Fig. 3. Effects of total volume of incubation medium on rooster testicular testosterone formation. The dispersed cell suspensions prepared via our described mechanical method were incubated in 1 ml, 2 ml, and 4 ml of incubation medium at 37°C for 4 hr. Data are expressed as mean ± SEM (triplicates from a single assay).

testosterone formation at the same doses of ovine LH. When testicular cells were incubated with 0.5 ng of ovine LH, testosterone amounts formed in 1.0 ml, 2.0 ml, and 4.0 ml of incubation medium were 13, 5.8, and 2.5 ng, respectively. Furthermore, cells incubated in 1.0 ml of incubation medium were observed to be the most sensitive.

Comparisons of LHs from various vertebrate species on rooster testicular testosterone formation

Ovine, porcine, duck, and chicken LH, as well as piscine GTH, produced parallel dose-related testosterone formation curves; however, other pituitary hormones (FSH, TSH, GH, and PRL) did not stimulate testosterone formation in the rooster testicular cells. As shown in Fig. 4, ovine LH was the most potent, with 0.1 ng/vial being sufficient to stimulate the formation of testosterone. Minimum effective doses of other LHs/GTHs required to elicit significant increases in testosterone formation were 0.2, 0.4, 1.0, and 10 ng for duck LH, porcine LH, chicken LH and grass carp GTH, respectively.

The LH content of duck pituitary glands

The LH content of duck pituitary glands in two age groups were determined by *in vitro* rooster testicular testosterone formation using duck LH as a reference hormone. As shown in Fig. 5, dose-related testosterone formation curves for different age groups were parallel to each other. The LH content of the pituitary glands of 70- and 250-day-old female ducks were calculated to be 3.5 μg and 22.5 μg of duck LH (with reference to ASIZ-dLH-1), respectively.

Changes in circulating LH levels of the duck in response to GnRH stimulation

Both basal and GnRH-injected LH levels

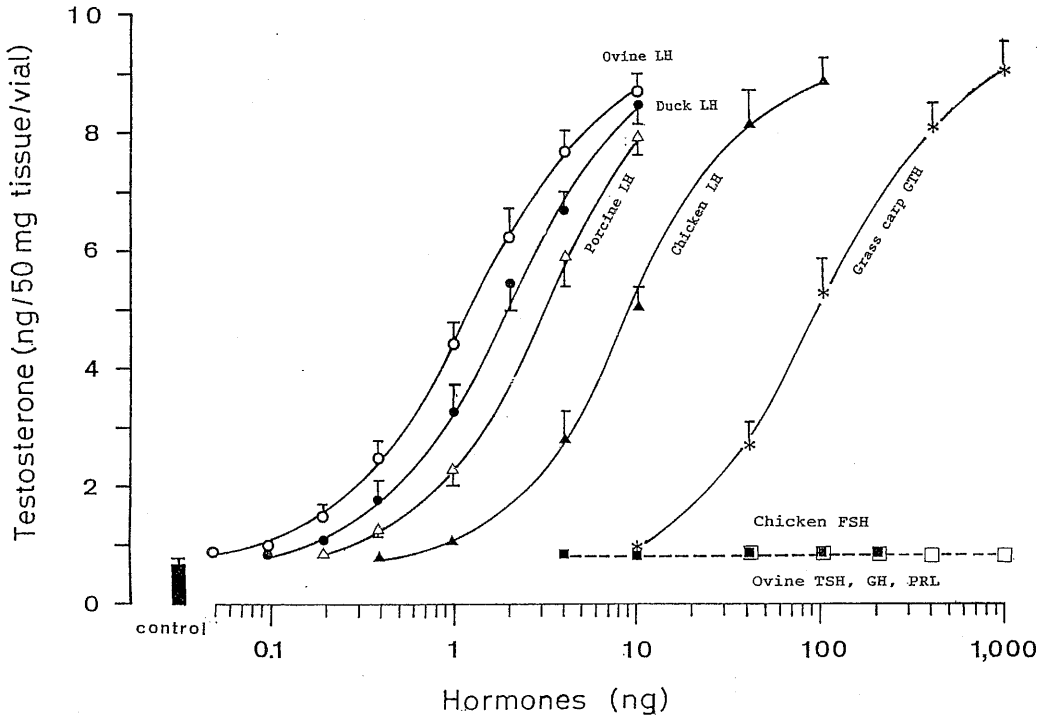

Fig. 4. Testosterone formations of rooster dispersed testicular cells in response to LHs from mammalian (ovine and porcine), avian (duck and chicken), and piscine (grass carp) GTH, as well as to other pituitary hormones (FSH, TSH, GH, and PRL). The data are expressed as mean ± SEM (triplicates from a single assay).

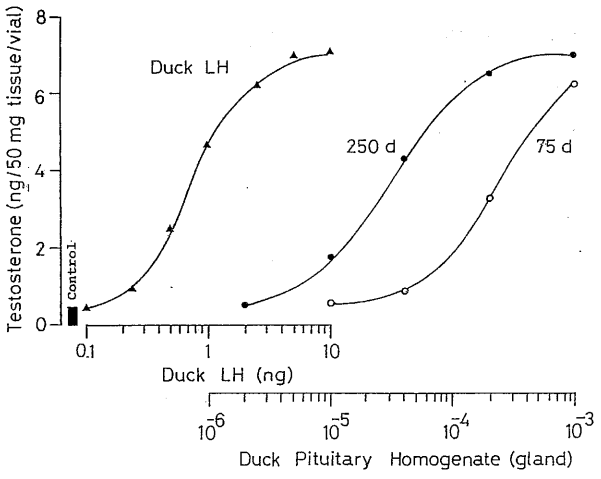

Fig. 5. Dose response of testosterone formation of duck pituitary gland extracts assayed by the rooster testicular system. Data are representative of assays from a single pituitary extract obtained from 75- and 250-day-old female ducks.

in the circulations of our studied ducks were successfully estimated via this system. Dose-related testosterone formation was induced when rooster testicular cells were incubated with various volumes of duck serum sampled at various time intervals following GnRH injection. These testosterone formation curves were parallel to that for duck LH (Fig. 6). Changes in LH levels in duck sera following GnRH injection are shown in Fig. 7; LH levels increased rapidly after injections of GnRH, reached their maximum at 10 min, then gradually decreased thereafter. In contrast, control LH levels (injected with saline only) did not increase during the 120 min sampling period.

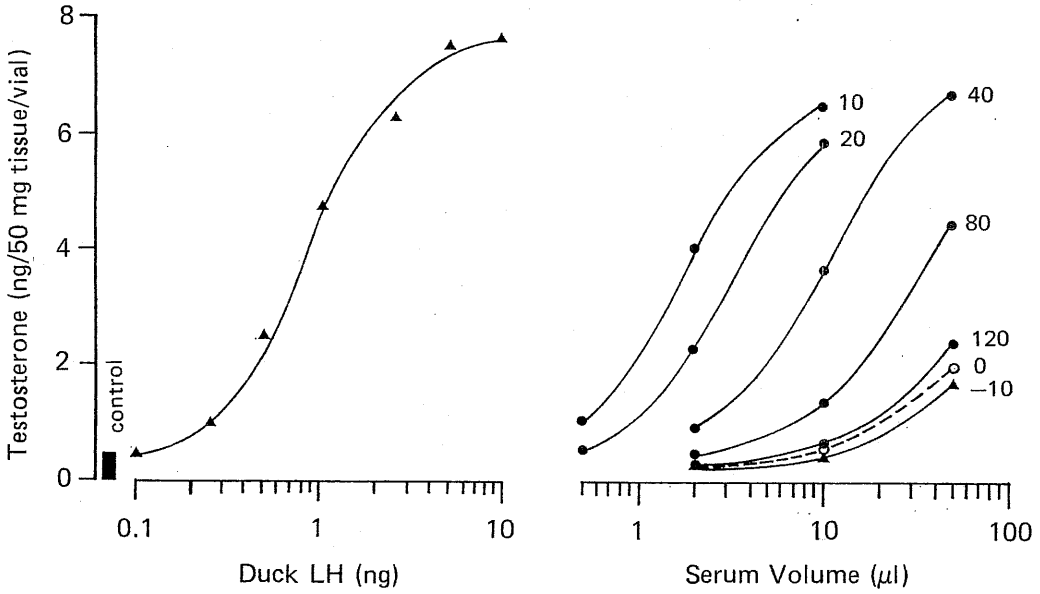

Fig. 6. Testosterone formation of rooster testicular cells in response to duck sera following injections of GnRH. Blood sampling time intervals were at −10, 0, 10, 20, 40, 80, and 120 min. Data are expressed as mean from a single assay of duplicate incubations. None of the dose-response curves are significantly non-parallel to each other ($p < 0.05$).

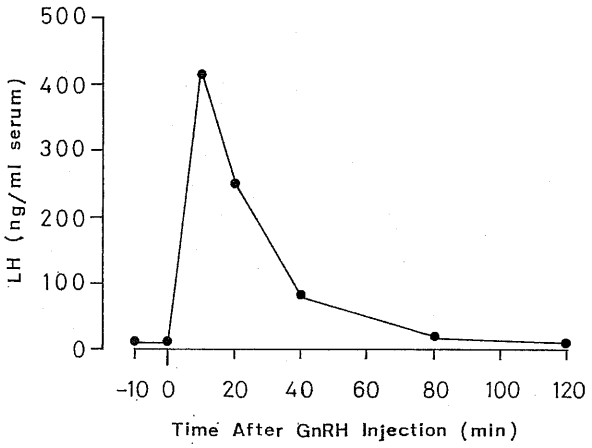

Fig. 7. LH levels of Tsaiya duck serum after GnRH stimulation. The LH concentrations of the duck sera were calculated from the dose response testosterone formation curves shown in Fig. 6. The control values remained constantly low during the 120 min sampling period.

DISCUSSION

This study established a simple and convenient method for preparing rooster dispersed testicular cells, as well as for setting up a sensitive *in vitro* bioassay for measuring both purified LHs from various vertebrate species and LH levels of duck pituitary and blood sera.

Our time course patterns revealed that the formation of testosterone by rooster dispersed testicular cells reached a plateau after four hours of incubation (Fig. 1); these patterns are similar to those resulting from rooster sliced testis preparations (Yu and Wang 1987). Dispersed cells incubated in 1.0 ml of incubation volume were more active in testosterone formation than those incubated in 2.0 and 4.0 ml of incubation medium. We also compared the testosterone formation activity of rooster testicular cells incubated in 5 ml polystyrene tubes (12 × 75

mm) and 20 ml counting vials (27 × 55 mm) under identical incubation conditions. Testosterone formation in the cells incubated in 5 ml polystyrene tubes was relatively low, and less reproducible as compared to those incubated in 20 ml counting vials. We also observed that testicular cells aggregated when incubated in 5 ml polystyrene tubes; in contrast, cells incubated in 20 ml counting vials remained well-dispersed during the 4 hr incubation period.

Dispersed rooster testis cells are highly responsive to LHs, but are unresponsive to other pituitary hormones (FSH, TSH, GH and PRL), therefore exhibiting strong LH specificity. As observed in this study, LHs from various vertebrate species and piscine GTH stimulated parallel dose-related testosterone formation by dispersed rooster testicular cells (Fig. 4). These results are comparable to those observed in rooster testicular slices, thus illustrating that both dispersed cells and the sliced tissues of rooster testes are suitable for assaying LHs from various vertebrate classes (Yu and Wang 1987).

The sensitivity of the dispersed rooster testicular cell system was 0.1 ng of NIADDK-oLH-25 (equivalent to 0.23 ng of NIH-LH-S1), or 0.4 ng for chicken LH (CANOMS124326) (Fig. 4). It is comparable to that of sliced testis preparations (Yu and Wang 1987), and more sensitive than that of minced rooster testes preparations (equivalent to 1 ng of NIH-LH-S1) (Ax 1978).

The preparation of duck LH antiserum and development of duck LH radioimmunoassay are not yet available. Therefore, the chicken LH radioimmunoassay system was used to assay the LH levels of duck serum (Tanabe *et al.* 1983). We previously isolated and purified duck LH possessing high biological activity traits; using this purified duck LH as a reference hormone for our *in vitro* bioassay, we successfully developed a rooster testis system for quantifying the LH levels of both duck pituitary gland and blood sera. We believe the establishment of an *in vitro* bioassay for measuring the circulating LH levels of ducks has not been previously reported.

Dose-related testosterone formation was induced by serial dilutions of duck serum samples, in addition, the formation was parallel to that of duck LH. These findings indicate that our established rooster testis testosterone formation system is a suitable and valid *in vitro* bioassay for measuring LH levels in duck serum. The maximum volume of duck serum used in this incubation system was 50 μl, and testosterone content before and after GnRH injection was measured at the range of 20 – 80 picogram (pg). The effect of serum testosterone in this assay system is thus negligible when compared with testosterone formation in incubated testicular cells stimulated by LHs. LH concentrations in duck blood serum increased rapidly within ten minutes following GnRH injection, then gradually decreased to basal levels (Fig. 7). The LH release pattern in ducks stimulated by GnRH observed in this study is comparable to those in chickens, rats, and other animals injected with GnRH, as measured either by *in vitro* bioassays or radioimmunoassays (Dufau *et al.* 1977, Ishii and Hattori 1984, Steiner *et al.* 1980, Lincoln 1985). The reproducibility and precision of this bioassay system are thus comparable to those employing mammalian testicular testosterone formation systems for measuring circulating LH levels in mammalian samples (Dufau *et al.* 1977, Van Damme *et al.* 1974, Steiner *et al.* 1980). The hormonal specificity, sensitivity, and reproducibility of the testosterone formation system of rooster dispersed testicular cells established in our investigation are thus valid for *in vitro* assays of LHs from duck pituitary gland and blood sera.

Acknowledgments: This study was sup-

ported by the National Science Council (NSC 76-0201-B001-05) and Academia Sinica, both of Taiwan, R.O.C. We are grateful to the National Hormone and Pituitary Program, NIADDK, the USDA Animal Hormone Program, USA, and Dr. S. Ishii for supplies of various pituitary hormones used in this study.

REFERENCES

Ax RL. 1978. Development and validation of a bioassay for chicken LH and application to chicken serum. Ph.D. thesis, Univ. Illinois, Urbana Champaign.

Bernier M, W Gibb, GJ Haour, R Collu, JM Saez, JR Ducharme. 1983. Studies with purified immature porcine Leydig cells in primary culture. Biol. Reprod. **29:** 1172-1178.

Ding YQ, I Huhtaniemi. 1989. Human serum LH inhibitors: behavior and contribution to *in vitro* bioassay of LH using dispersed mouse Leydig cells. Acta Endocrinol. (Copenhagen) **121:** 46-54.

Dufau ML, GD Hofgen, AL Goodman, KJ Catt. 1977. Bioassay of circulating luteinizing hormone in the rhesus monkey: comparison with RIA during physiological changes. Endocrinology **100:** 1557-1565.

Dufau ML, DR Mendelson, KJ Catt. 1974. A highly sensitive *in vitro* bioassay for luteinizing hormone and chorionic gonadotropin: testosterone production by dispersed Leydig cells. J. Clin. Endocr. Metab. **39:** 610-631.

Dufau ML, R Pock, A Neubauer, KJ Catt. 1976. *In vitro* bioassay of LH on human serum: the rat interstitial cell testosterone (RICT) assay. J. Clin. Endocr. Metab. **42:** 958-969.

Farmer SW, A Suyama, H Papkoff. 1977. Effect of diverse mammalian and nonmammalian gonadotropins on isolated rat Leydig cells. Gen. Comp. Endocrinol. **32:** 488-494.

Finney DJ. 1964. Statistical Method in Biological Assay. New York: Hafnet Publishing Co., pp. 58-138.

Glenn SD, WK Liu, DN Ward. 1981. Characteristics of hybrids of ovine LH and human glycoprotein hormone subunits in rat and chicken *in vitro* test systems. Biol. Reprod. **25:** 1027-1033.

Haour F, JM Saez. 1977. Regulation by hCG of gonadotropin receptors in testicular Leydig cells: Evidence for a down regulation. Mol. Cell. Endocrinol. **7:** 17-24.

Ishii S, A Hattori. 1984. Avian gonadotropin-releasing hormones. *In* "Evolutionary Aspects of Gonadotropins." ed. Institute of Endocrinology, Gunma University. pp. 79-91.

Jenkeins N, JP Sumpter, BK Follet. 1978. The effects of vertebrate gonadotropins on androgen release *in vitro* from testicular cells of Japanese quail and a comparison with their radioimmunoassay activities. Gen. Comp. Endocrinol. **35:** 309-321.

Licht P, CJ Muller, HW Tsui. 1976. Effects of mammalian and nonmammalian gonadotropins on androgen production by minced rabbit testis. Biol. Reprod. **14:** 194-201.

Licht P, H Papkoff, SW Farmer, CH Muller, HW Tsui, D Crews. 1977. Evolution of gonadotropin structure and function. Recent. Prog. Horm. Res. **33:** 169-248.

Lincoln D. 1985. Hypothalamic pulse generator. Recent. Prog. Horm. Res. **41:** 369-396.

Maung ZW, BK Follett. 1977. Effects of chicken and ovine luteinizing hormone on androgen release and cyclic AMP production by isolated cells from the quail testis. Gen. Comp. Endocrinol. **33:** 242-253.

Ramachandran J, MR Sairam. 1975. The effects of interstitial cell-stimulating hormone, its subunits, and recombinants on isolated rat Leydig cells. Arch. Biochem. Biophys. **167:** 294-300.

Steiner RA, AP Peterson, JYL Yu, H Conner, M Gilbert, B Terpening, WJ Bremner. 1980. Ultradian luteinizing hormone and testosterone rhythms in the adult male monkey, *macaca fascicularis*. Endocrinology **107:** 1489-1493.

Tanabe Y, T Yang, T Nakamura. 1983. Steroid hormone synthesis and secretion by testes, ovary, and adrenals of embryonic and postembryonic ducks. Gen. Comp. Endocrinol. **49:** 144-153.

Van Damme MP, DM Robertson, E Diczfalusy. 1974. An improved *in vitro* bioassay of luteinizing hormone (LH) activity using mouse Leydig cell preparations. Acta Endocrinol. (Copenhagen) **77:** 655-671.

Yu JYL, TY Chang, HK Hsu, CF Liao, WCM Wan. 1981. Androgen/testosterone synthesis by the dissociated testicular cells from mice of different ages in response to rat LH stimulation *in vitro*. Bull. Inst. Zool., Academia Sinica **20:** 57-65.

Yu JYL, JJ Liaw, CS Lin, MC Chen. 1990. Changes in levels and ratios of androgen, estradiol-17β and progesterone in peripheral plasma of dairy cows during the first 60 days of pregnancy. Bull. Inst. Zool., Academia Sinica **29:** 213-222.

Yu JYL, ST Shen. 1989. Isolation of pituitary glycoprotein gonadotropins from the grass carp (*Ctenopharyngodon idell*). Fish Physio. Biochem. **7:** 177-183.

Yu JYL, ST Shen, YC Wu, SH Chen, CT Liu. 1991. Gonadotropin specificity and species diversity of gonadal steroid hormone formation in fish. Bull. Inst. Zool., Academia Sinica Monograph **16:** 61-88.

Yu JYL, TK Tang, TM Lin, ST Shen. 1987. Isolation and purification of pituitary luteinizing hormone from the duck. *In* Proceedings of the First Congress Asia Oceania Society of Comparative Endocrinology, Nagoya, Japan. pp. 132A-132B.

Yu JYL, LM Wang. 1987. Comparative effects of diverse vertebrate gonadotropins on androgen formation *in vitro* from testes of roosters and mice. Biol. Reprod. **18:** 55-64.

Yu JYL, LM Wang, ML Fei. 1984. Comparative effects of mammalian gonadotropins on androgen formation *in vitro* from mouse testis interstitial cells. Bull. Inst. Zool., Academia Sinica **23:** 81-91.

鴨血清黃體促素離體生物活性測定法之建立：公雞睪丸睪固酮生成系統

沈 三 泰　余 玉 林

本研究目的是建立公雞分散睪丸細胞之睪固酮生成系統，作爲純化之黃體促素及鴨血清黃體促素之離體生物活性測定法。睪丸組織取自3-4月齡公雞，將5公克之組織，置於50毫升Medium 199，以塑膠滴管及玻璃注射筒分離睪丸細胞，直到形成均勻細胞懸浮液。細胞於37°C預先培養半小時後，加入黃體促素、腦下垂體萃取液，或鴉血清，繼續培養於37°C恆温水浴振盪槽4小時（每分鐘振盪100次），並連續通以混合氣（95% O_2－5% CO_2）。以放射性免疫分析法測定培養液中睪固酮含量。

哺乳類和鳥類黃體促素，與魚類性腺促素皆可引起劑量相關之睪固酮生成，而其他腦下垂體相關激素（濾胞促素、甲狀腺促素、生長激素及泌乳素）並不能促進本系統睪固酮生成。本測定法之最低靈敏度爲0.1ng羊黃體促素(NIADDK-oLH-25）或0.2ng鴨黃體促素(ASIZ-dLH-1)。鴨隻血清可引起劑量相關之睪固酮生成，與鴨黃體促素所引起劑量相關之睪固酮生成曲線互相平行。鴨隻注射性釋素所引起血液中黃體促素濃度變化，亦可以本法測定之。因此，所建立公雞睪丸分散細胞之睪固酮生成，可作爲哺乳類及鳥類黃體促素，以及鴨腦下垂體與血液中黃體促素之離體生物活性定性與定量測定法。

Bull. Inst. Zool., Academia Sinica **32**(3): 204-213 (1993)

Regulation of Cell Growth and Alteration of Gene Expression in Human Hepatoma Cells by a Carp Maternal Gene

Pei-Yen Yeh and Fu-Hsie Yew

Department of Zoology, National Taiwan University
Taipei, Taiwan 107, R.O.C.

(Accepted March 31, 1993)

Pei-Yen Yeh and Fu-Hsie Yew (1993) Regulation of cell growth and alteration of gene expression in human hepatoma cells by a carp maternal gene. *Bull. Inst. Zool., Academia Sinica* **32**(3): 204-213. A carp maternal cDNA library was used to transfect tumor-like tilapia ovary cells in culture, and a plasmid was subsequently rescued from a single cell clone. Transfection of the rescued plasmid into human hepatoma cells caused them to lose their tumorous characteristics; they were unable to pile up and failed to grow in soft agar. Moreover, we found that a co-culture of transformed and parental cells, both tilapia ovary and human hepatoma cells, could effectively suppress the tumorous phenotypes of the parental cells. The screening of several cellular proteins by western blot immunostaining showed that α-fetoprotein and oncogenic proteins, *ras*, and *abl* were reduced in transformed cells; in addition, phosphorylated proteins with molecular weights corresponding to $pp60^{src}$ and EGF receptor were decreased. However, the tumor suppressor gene p53 product and a matrix protein collagen type IV were enhanced. Our results suggest that this maternal gene may be directly involved in cell growth and differentiation control.

Key words: Maternal gene, Growth regulation, Hepatoma cell.

In vivo, most somatic cells reside in a quiescent state. In the absence of growth factors, or as a result of their depletion, cells in culture are usually restricted to the G1 stage and are unable to divide. During early embryogenesis, however, blastomeres undergo rapid cell division, and in early embryos the cell cycle consists of only S phase and mitosis until reaching midblastula stage (Newport and Kirschner 1982). Previous studies on early embryogenesis have suggested that maternal factors which are present in eggs may overcome cell growth restrictions in somatic cells. Several growth factors and peptides that are homologous to oncoproteins have been identified in the maternal genes or proteins or mature eggs; these factors have been shown to affect embryonic development and blastoderm induction (for a detailed review, see Jessell and Melton 1992). In xenopus eggs, the maternal factor Vg1 which is homologous to transforming growth factor β (TGFβ) can cooperate with FGF to induce mesoderm formation (Weeks and Melton 1987, Kimelman and Kirschner 1987); however, in culture TGFβ has an inhibitory effect on myogenesis (Massague *et al.* 1986) as well as multiple effects on cell growth and differentiation in a cell type-dependent manner (Barnard *et al.* 1990, Rizzino 1988). The mechanisms and functions of these growth factors are still uncertain, and cell response