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SHORT REPORTS

A SENSITIVE *IN VITRO* BIOASSAY OF LUTEINIZING HORMONE USING ROOSTER TESTICULAR PREPARATIONS[1]

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Ling-Mei Wang and J. Yuh-Lin Yu (1982) A sensitive *in vitro* bioassay of luteinizing hormone using rooster testicular preparations. *Bull. Inst. Academia Sinica* 21(2): 171-174. A simple and sensitive *in vitro* biological assay of luteinizing hormones (LH's) from both mammalian and avian species was developed using rooster testicular slice preparations. Incubation of 4-5 testicular slices weighing about 100 mg/vial was carried out at 37°C for 4 hrs in Medium 199 (pH 7.40) under continuous aeration of 95% O_2-5% CO_2. Androgen formation of such preparation was highly responsive to LH stimulations. The lower detection limit was 0.1 ng and 0.5 ng, respectively for ovine LH-S22 and chicken LH-AGCHDS-11-2312A. Such system appears to be considerably more sensitive than the currently available LH bioassays using avian testicular preparations.

The development of the technique in isolating and dispersing the interstitial cells from mouse testis has been achieved in our labarotory, for *in vitro* study of steroidogenesis, and for use as a heterologous bioassay of gonadotropins[8,9,10]. However, the interstitial cells isolated from rat testis were only useful for bioassay of gonadotropins from mammalian species[4]. Chicken testes have also been used as an *in vivo* bioassay of pituitary gonadotropins from mammalian and avain species[2,6]. The purpose of the present study was to establish an *in vitro* technique using rooster testes, for convenient bioassay of LH's from both mammalian and nonmammalian species. LH-stimulated formation capacity by both dispersed testicular interstitial cells and testicular slices from roosters was used as an index for the bioassay. The androgen production activity by dispersed interstitial cells from mouse testis was also compared.

MATERIALS AND METHODS

Six-week-old, male mice (IRC, U. S. A.), were purchased from the National Laboratory Animal Resources, National Taiwan University. Four to six-month-old chicken, male, Hybro strain (Euribrid Co., Netherland), were purchased from the Tongin Co., Taipei.

The method to disperse interstitial cells from chicken and mouse testis is a modified procedure reported by Dufau *et al.*[3], and Jenkins *et al.*[7]. Following sacrifice of the animal, testis was cut into pieces and transferred to scintillation vial containing preincubation medium (Medium 199 with Hank's salts, L-glutamine; 25 mM HEPES; penicilin, 10,000 units/100 ml; streptomycin, 5 mg/100 ml; 0.2% bovine serum

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albumin: and 10% sodium bicarbonate, 1 ml/100 ml; pH 7.40) (1 mg collagenase+1 g testis+2 ml preincubation medium+0.5 mg trypsin inhibitor). Incubation was then performed at 37°C by shaking at 100 cycles/min for 30 min with the vial capped. The dispersed cells were washed twice by fresh medium and then suspended in incubation medium (preincubation medium+0.125 mM methylisobutyl xanthine+sodium heparin, 10,000 USP units/100 ml). Incubation was performed at 37°C (for chicken testis) or 34°C (for mouse testis), shaken at 100 cycles/min in a Dufnoff incubator, under continuous aeration (95% O_2-5% CO_2). Various dose of LH's were incubated with the interstitial cells. Following incubation, the supernatants were collected and stored at −25°C until assay for androgen.

The testicular slices preparation from roosters is described briefly as follows: Roosters were decapitated, testes were excised, and immersed into aerated preincubation medium. Testes were decapsulated, weighed, and cut into slices (about 20 mg/slice). Four to five slices of testes (about 100 mg), were pooled, weighed, and placed into a scintillation vial containing medium. The vials were capped and preincubated at 37°C, shaking at 50-60 cycles/min for 60-90 min. After preincubation was stopped, the testicular slices recieved fresh medium (preincubation medium +0.125 mM methylisobutyl xanthine) and various doses of LH. Incubation was perfomed under continuous aeration at 37°C, shaken at 100 cycles/min. Following incubation, the suspensions were collected and stored at −25°C for assay of androgen.

The total androgen was determined by radioimmunoassay using ^{3}H-testosterone and testosterone antiserum as described previously[8].

RESULTS AND DISCUSSION

The viability of the mouse testicular cells determined by trypan blue staining was approximately 80%. These cells produced androgen in a dose-response relationship following ovine LH stimulation. Positive linear androgen formation (2-20 ng/0.35×10^6 cells) was obtained in response to stimulation of ovine LH-S22 ranging from 0.1 to 1.0 ng. Such data are comparable to those reported by previous workers[1,3].

The dispersed interstitial cells from rooster testis did not promote androgen production following stimulation with ovine LH-S22 ranging from 0.1 to 10 ng, although the viability being similar to that of the mouse testicular cells. Consistent negative results were obtained from four separate incubation experiments. Attemps to develop the bioassay using dispersed chicken testicular interstitial cells have been unsuccessful[1]. Such apparent species difference between mouse and rooster testis remaines unclear.

An incubation system using sliced rooster testis was subsequently developed. A dose-response relationship of androgen formation in responding to both ovine and chicken LH's was obtained (Fig. 1). The lower detection limit was 0.1 ng and 0.5 ng, respectively for ovine LH-S22 and chicken LH-AGCHDS-11-2312A.

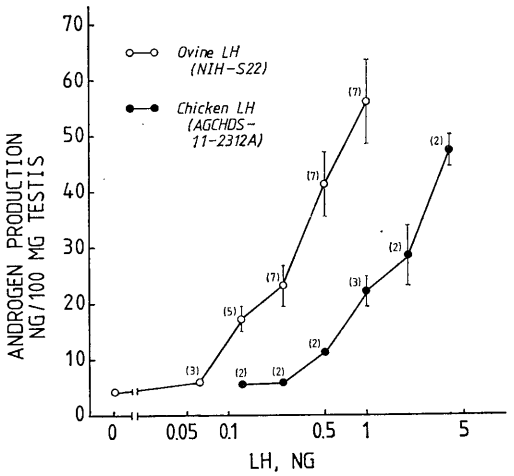

Fig. 1. Dose-response curves of rooster testicular slices in response to ovine LH-S22 (NIH, U. S. A.) and chicken LH-AGCHDS-11-2312A (Susumu Ishii). Each tube contained 5 testicular slices weighing about 100 mg and was incubated at 37°C for 4 hrs. The numbers in the parentheses denote the numbers of incubation experiments. The data are expressed as mean±SEM.

It was reported by other researchers that the minimal amount of ovine LH-S18 to promote androgen production was 600 pg/assay tube, using the minced rooster testis[1,5]. Follett and his colleagues[7] developed a collagenase-dispersed interstitial cells system from mature quail testis and reported that the sensitivity for ovine LH-S19 was 2 ng/assay tube. The biological potency of ovine LH-S22 used in the present study was similar to those used by previous researchers[1,5,7]. It thus appears that the rooster testicular slices system developed in the present study is considerably more sensitive than the currently available bioassay using avian testis[1,5,7], providing a useful *in vitro* bioassay for LH's from mammalian and avian species.

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以雞睪丸組織建立黃體生成激素之離體生物測定法

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本研究係利用鷄之睪丸組織薄片建立離體生物測定法，以定量哺乳類及鳥類之黃體生成激素 (Luteinizing hormone, LH) 之濃度。以 4～5 片睪丸組織（約 100 毫克/管），與黃體生成激素一起培養於 Medium 199 (pH 7.40)，凡 4 小時；培養期間連續通以混合氣 (95% O_2-5% CO_2)，並維持在 37°C 水浴中。雄性素 (Androgen) 之產生則與加入之黃體生成激素呈劑與量之正相關。最低可測得之羊與雞之黃體生成激素 (Ovine LH-22; Chicken LH-AGCHDS-11-2312A)，分別爲 0.1 ng 與 0.5 ng。本法之測定敏感度遠高於其他以鳥類睪丸做爲黃體生成激素之離體生物測定法。