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Developmental changes of plasma inhibin, gonadotropins, steroid hormones, and thyroid hormones in male and female Shao ducks

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Abstract

Plasma samples from developing male and female Shao ducks were assayed for immunoreactive (ir-) inhibin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), steroid hormones, and thyroid hormones. In the male, plasma ir-inhibin significantly increased between 75 and 155 days posthatch, and then decreased slightly at day 165. Plasma FSH of male ducks decreased from day 35 to day 55, followed by progressive elevation until day 95. Plasma FSH of male ducks fell significantly at days 135 and 165, while plasma ir-inhibin rose to high level. In female ducks, plasma ir-inhibin remained low until the start of lay, and thereafter significantly increased at day 135. Plasma FSH fluctuated before day 95 and significantly rose at day 115, and decreased thereafter. In males, plasma LH did not vary significantly before day 135, however, plasma testosterone significantly increased from day 95 onwards. No changes in plasma LH were observed during development of female ducks. Plasma estradiol-17β gradually increased reaching a peak level at day 135. Plasma progesterone did not vary significantly before day 135 and thereafter significantly increased. Both sexes showed a similar pattern in changes of plasma thyroid hormones during sexual development. There was a significant increase in plasma thyroxine (T_4) at day 95, thereafter decreased. Plasma triiodothyronine (T_3) was at high level at the earlier stage of development and significantly decreased at day 55. Significant increase in plasma T_3 in male and female ducks was observed at 135 and 115 days, respectively. In conclusion, these results demonstrated that the rise in inhibin is correlated with age of sexual maturity in the female while the rise in inhibin significantly precedes sexual maturity in the male. There was a progressive increase in plasma steroid hormones towards sexual maturity, and there was no sex difference in the time course of thyroid hormones.

Keywords: Inhibin; FSH; Steroid hormones; Thyroid hormones; Duck

1. Introduction

Reproductive hormones have a key role in the differentiation and maturation of sexual organs with other hormones' interrelationship. Gonadotropins stimulate the development of gonads and are regulated in a negative feedback manner by gonadal steroids. Inhibin is a dimeric gonadal glycoprotein which consists of an α-subunit and a β_A- or β_B-subunit, and selectively suppresses follicle-stimulating hormone (FSH) secretion from the anterior pituitary gland. Homodimers of β-subunits called activin stimulate FSH secretion (de Jong, 1988; Ying, 1988). In addition to the regulation of FSH secretion, it has been clearly shown that inhibin and activin have paracrine/autocrine effects on the gonads (Findlay, 1993). Secretion of bioactive inhibin by chicken granulosa cells in vitro has been reported (Akashiba et al., 1988; Tsonis et al., 1988). In the hen, as in mammals, the ovary was shown to be the primary source

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of immunoreactive (ir-) inhibin (Vanmontfort et al., 1992). Removal of large follicles leads to a rise in plasma FSH levels, indicating a possible endocrine role of inhibin in FSH regulation in the chicken (Johnson et al., 1993). In previous studies, inhibin/activin α- and β_A-subunit messenger RNA (mRNA) have been demonstrated to be expressed in the chicken ovary (Chen and Johnson, 1996a; Onagbesan et al., 2004; Wang and Johnson, 1993), and Northern blot hybridization has revealed differential expression of α- and β_A-subunit mRNA during preovulatory follicle development (Chen and Johnson, 1996b). In addition, inhibin/activin dimers have been detected in the granulosa and theca layers, and have shown differential changes during preovulatory follicle development in the hen (Lovell et al., 1998). Ir-inhibin was present in plasma and gonads of the chicken, and varied with embryonic development (Rombauts et al., 1992). Interestingly, inhibin/activin subunits were localized in the preovulatory follicles in ducks (Yang et al., 2001a) and in the duck fetal ovary (Yang et al., 2001b).

Inhibin has been demonstrated to have a role in the modulation of the timing of puberty in sheep (O'shea et al., 1993). A negative relationship between FSH and inhibin was also found in the postnatal rat (Culler and Negro-Vilar, 1988). The relationship between pituitary gonadotropins and gonadal inhibin was characterized from hatch to maturity in chickens (Johnson and Brooks, 1996; Vanmontfort et al., 1995). Both studies found a negative relationship between inhibin and FSH in the female chickens that appeared to become functional at sexual maturity. Thyroid gland is a target organ for sex hormones in mammals and human beings (Banu et al., 2001; Furlanetto et al., 1999; Miki et al., 1990). Estradiol negatively influences thyroid gland function (Bagchi et al., 1984). *Similarly*, there is a negative relationship between the ovary and the thyroid gland during sexual maturation of the domestic hen in which increasing concentrations of plasma steroids, progesterone, and estradiol were accompanied by decreasing levels of plasma iodothyronines, triiodothyronine, and thyroxine (Sechman et al., 2000). In the present study, we examine the correlation of the gonadal and thyroid gland functions by paralleling measuring sex hormones and thyroid hormones.

Little is known concerning the initiation of puberty in ducks. Inhibin-related protein may be important factors with respect to puberty. Limited data have reported about inhibin/activin of other avian species except chicken. To investigate the relationship between inhibin and gonadotropin, and the possible effect of thyroid hormones on gonadal function during the development of ducks, the present study examined changes in circulating inhibin, gonadotropin, steroid hormones, and thyroid hormones in both male and female Shao ducks during development.

2. Materials and methods

2.1. Animals

This study was carried out on Shao ducks (Chinese local strain). Shao ducks are characterized by small body size, high egg production, and early maturity (around 160 and 135 days in male and female, respectively). The ducks were raised from hatch to 180 days of age in experimental room provided with a piece of water area in a farm. The ducks were leg banded for identification and had free access to water and food. Before 60 days of age, the ducks were housed under natural lighting, while from 60 days of age onwards, the ducks were maintained on a lighting schedule for 24 h. Blood samples were collected from 10 ducks of both sexes at 1, 15, and 35 days after hatch, and subsequently at 20-day intervals until 155 days of age. For males, a further sample was collected at 165 days of age. Blood samples (2 ml) were taken by cardiac puncture at birth and by venipuncture from the wing vein afterwards. Blood was collected into heparinized tubes, the plasma was separated by centrifugation and stored at −20 °C until assayed for hormones. Values for some hormones at earlier time points are not presented because insufficient plasma was available for assay. The ducks were weighed and egg production records were kept for females. Histological characteristics of testes at different ages help us to determine the time of onset of puberty in male (numerous sperms were seen in the testis of ducks of 120 days of age, indicating that the beginning of spermatogenesis was around this time point; data not shown), whereas in female, we used the material of egg production record.

2.2. Radioimmunoassay (RIA) of ir-inhibin, FSH, LH, T_3, T_4, progesterone, estradiol-17β, and testosterone

Concentrations of ir-inhibin in plasma were measured in triplicate (50 μl per sample) using rabbit antiserum against bovine inhibin (TNDH-1) and ^{125}I-labeled 32-kDa bovine inhibin as described previously (Hamada et al., 1989) and validated for use in ducks (Yang et al., 2001a,b). Results were expressed in terms of 32-kDa bovine inhibin. The intra- and inter-assay coefficients of variation were 5.7 and 8.9%, respectively.

Highly purified chicken FSH (AGCQSQ 111232D) and anti-chicken FSH antiserum raised in a rabbit were kindly supplied by Dr. S. Ishii (Waseda University, Japan). The FSH radioimmunoassay has been modified from that originally described by Sakai and Ishii (1980). Iodination was accomplished by chloramine-T procedure. The standard used was chicken gonadotropin standard fraction (AGC112B). Results were expressed in terms of chicken FSH (AGCQSQ 111232D). The intra- and inter-assay coefficients of variation were 6.2 and 7.0%, respectively. Plasma concentrations of LH were

determined in duplicate by a double-antibody RIA system using ^{125}I-labeled radioligands, chicken LH preparation (AGMS1122F, provided by Dr. S. Ishii, Waseda University, Japan), as described previously (Mauget et al., 1994). Antiserum against chicken LH (HAC-CH27-01RBP75) was kindly provided by Dr. K. Wakabayashi, Guma University, Japan. Results were expressed in terms of nanograms of AGMS1122F LH per milliliter of plasma. The intra- and inter-assay coefficients of variation were 5.4 and 8.2%, respectively.

Triiodothyronine (T_3) and thyroxine (T_4) were determined using the commercially available RIA kits (Shanghai Bioproducts Institute, Shanghai, China). The intra- and inter-assay coefficients of variation were 6.3, 5.1%, and 11.2, 9.9% for T3 and T4, respectively.

Progesterone, estradiol-17β, and testosterone were extracted from plasma using petroleum ether. Plasma concentrations of progesterone, estradiol-17β, and testosterone were determined using commercially available RIA kits (Shanghai Bioproducts, Shanghai, China). The intra- and inter-assay coefficients of variation were 5.6 and 7.4 for progesterone, 5.2 and 7.6 for estradiol-17β, and 4.8 and 8.6 for testosterone, respectively.

2.3. Statistics

Values are presented as means ± SEM. Statistical differences were assessed by repeated measures analysis of variance followed by Duncan's multiple range test (General Linear Models, SAS, 1987). Canonical correlation analysis was performed to measure correlation between inhibin and FSH and statistical significance was obtained using Wilks' Lambda test. A probability value (P) of less than 0.05 was considered to be significant.

3. Results

Changes in plasma concentrations of ir-inhibin and FSH in male and female Shao ducks at different developmental stages are shown in Fig. 1. In male ducks, plasma concentrations of ir-inhibin increased significantly from day 75 reaching a peak level on days 135 and 155 compared to earlier time points and then slightly decreased at day 165 posthatch. The rise in inhibin was correlated with age of sexual maturity in the female while the rise in inhibin significantly preceded sexual maturity in the male.

Changes of plasma concentrations of LH and testosterone in male ducks are depicted in Fig. 2. No significant changes were observed between day 15 and 135 except a small elevation at day 95. Plasma concentrations of LH significantly increased from day 135 reaching the highest value at day 165 posthatch. Plasma concentrations of testosterone progressively increased during the whole experimental period. In female ducks,

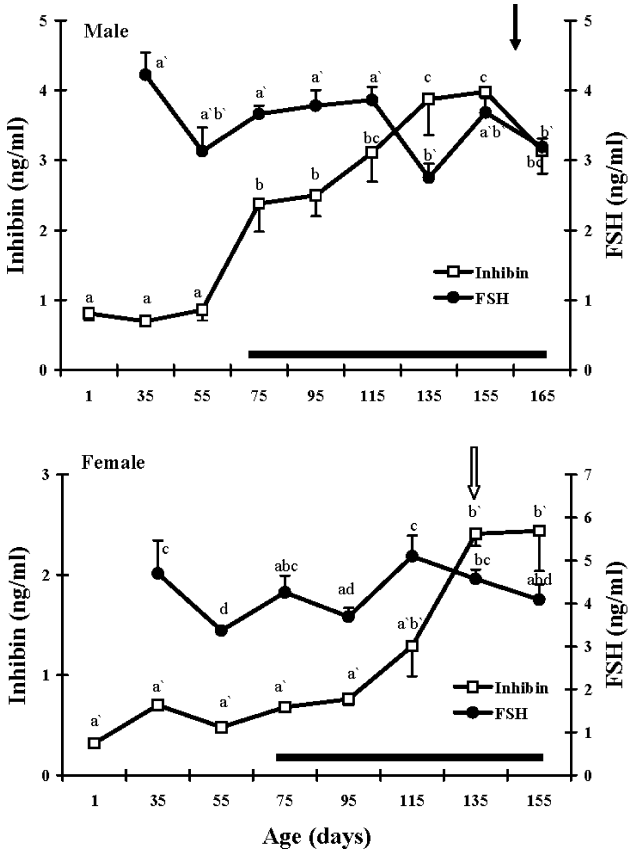

Fig. 1. Changes of plasma levels of ir-inhibin and FSH in male and female ducks at various developmental ages. Different letters indicate significant difference ($P < 0.05$) among time points within a hormone. Each point represents the mean ± SEM of six to eight animals. Horizontal bars denote constant lighting for 24 h and arrows denote the time of puberty.

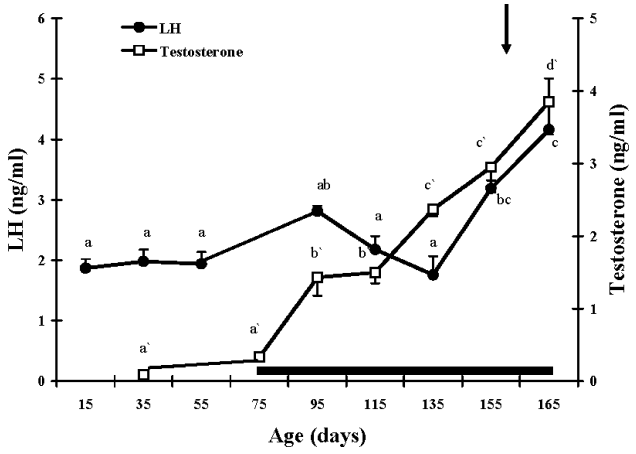

Fig. 2. Changes of plasma levels of LH and testosterone in male ducks at different developmental stages. Different letters indicate significant difference ($P < 0.05$) among time points within a hormone. Each point represents the mean ± SEM of seven animals. Horizontal bar denotes constant lighting for 24 h and arrow denotes the time of puberty.

developmental changes of plasma concentrations of LH, progesterone, and estradiol-17β are illustrated in Fig. 3. Overall, there was no significant change in plasma LH

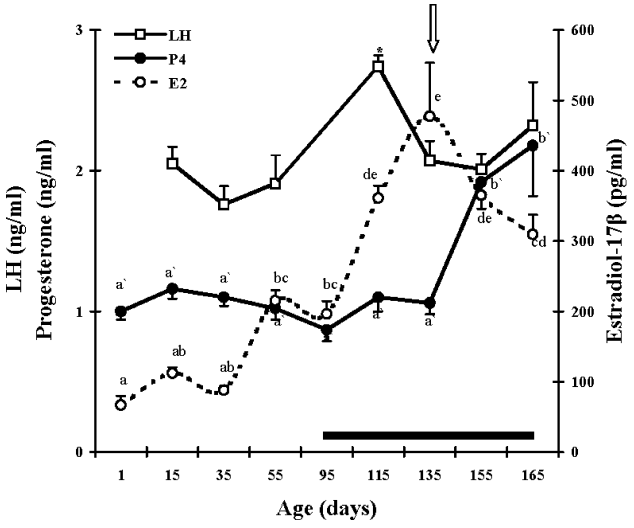

Fig. 3. Changes of plasma LH, estradiol-17β, and progesterone in female ducks at various developmental stages. Different letters indicate significant difference ($P < 0.05$) among time points within a hormone. Each point represents the mean ± SEM of seven animals. *$P < 0.05$, compared with other time points within the same hormone. Horizontal bar denotes constant lighting for 24 h and arrow denotes the time of puberty.

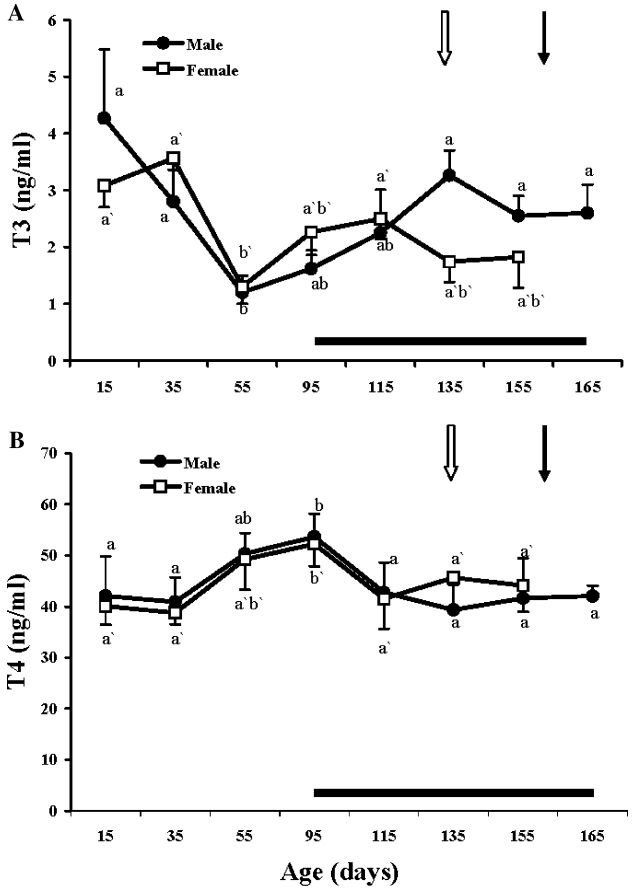

Fig. 4. Changes of plasma levels of T_3 (A) and T4 (B) in male and female ducks from 15 days of age to sexual maturity. Different letters indicate significant difference ($P < 0.05$) among time points within a hormone. Each point represents the mean ± SEM of five animals. Horizontal bars denote constant lighting for 24 h and arrows denote the time of puberty in male (black arrow) and female (white arrow).

levels other than one peak on day 95. Plasma concentrations of estradiol-17β gradually increased reaching a peak at day 135 and decreased thereafter. On the other hand, there was no significant change in progesterone level before day 135. Thereafter, plasma concentrations of progesterone significantly increased from day 135 onwards.

The developmental patterns of concentrations of plasma T_3 and T_4 for both sexes are shown in Fig. 4. Both males and females showed a similar pattern in the changes of plasma concentrations of T_3 and T_4. Plasma concentrations of T_4 significantly rose at day 95 compared to earlier time points in both sexes. Thereafter, plasma concentrations of T_4 decreased and maintained at relatively low values. Plasma concentrations of T_3 significantly decreased from earlier time points to day 55 and reached a significant trough at day 55 in both sexes. Thereafter, plasma concentrations of T_3 progressively increased and reached a significant peak at day 115 in females and at day 135 in males, respectively.

4. Discussion

In the present study, our main findings are: (1) circulating levels of ir-inhibin in males significantly elevated with development, (2) circulating levels of ir-inhibin in females significantly increased from 115 days of age onwards, and the inverse relationship between inhibin and FSH was in existence around this time points, (3) circulating levels of LH in males were significantly increased from 135 days onwards with progressive significant increase in plasma testosterone, whereas there was only one significant peak in LH level at day 115 in females with peak level in estradiol-17β at day 135 and in circulating level of progesterone at the beginning of laying, and (4) both sexes showed the same pattern of change in thyroid hormones.

Plasma ir-inhibin in both sexes was increased with the development of age. The main component in these increased ir-inhibin may be the inhibin α-subunit. Because the radioimmunoassay system used in the present study has limited specificity as previously reported (Kaneko et al., 1995), assessment of circulating ir-inhibin includes intact inhibin as well as free α-subunit. In rats, postnatal total mRNA levels of the three inhibin/activin subunits declined with age, with inhibin β_A levels decreasing most rapidly, and the inhibin α-subunit being the most abundant at every stage examined (Meunier et al., 1988).

Combined above observations, we propose that the increase of ir-inhibin is due to, especially in male ducks, the increase of production rate of free inhibin α-subunit

not intact bioactive inhibin in gonads before puberty, whereas around the time of puberty intact bioactive inhibin was increased with gonadal development stimulated by the preceded rise in circulating FSH. The latter part of our hypothesis is supported by these findings in females that the significant rise in circulating FSH at 115 days preceded the significant rise in circulating ir-inhibin at 135 days as has been demonstrated in the chicken (Johnson and Brooks, 1996). In males, there are relatively high plasma FSH levels in earlier times compared to later times, which may also be contributed to the progressive increase of plasma ir-inhibin before puberty.

As mentioned above, the main part of the progressive increase in plasma ir-inhibin in males may be the free inhibin α-subunit secreted by testis. The α-subunit may play as an autocrine and paracrine regulator on continuous development of the testis as has been demonstrated in mammals (Hsueh et al., 1987; Lin et al., 1989). In males, the progressive increase in plasma levels of inhibin is consistent with the results of previous studies in chicken (Vanmontfort et al., 1995) but contrasts with the decline observed in the male rat (Rivier et al., 1988) and ram (Schanbacher, 1988). Contrarily, in females before puberty, plasma concentrations of inhibin remained low, indicating that small white follicles in immature ovary secreted limited amount of inhibin. Supporting the present suggestion, Vanmontfort et al. (1994) have reported that when ovarian regression was induced by feed withdrawal, plasma concentrations of ir-inhibin decreased to basal level accompanying by an increase number of small white follicles. At peripuberty, yolk contained follicles were developed and the ovarian follicular hierarchy was gradually formed. Then, the granulosa layer of yolk contained follicle secreted great deal of intact inhibin resulting in significant rise in circulating inhibin.

From 115 days onwards, plasma concentrations of ir-inhibin in females were significantly increased compared to earlier times while plasma concentrations of FSH fell to basal levels from the maximum level at 115 days. Based upon these findings, it is likely that the FSH–inhibin feedback system is established in the female ducks at the time of sexual maturation (around 135 days) and thereafter. In avian species, a functional negative feedback between inhibin and FSH has been observed in adult hens (Johnson et al., 1993). Additional support for a negative feedback function of inhibin on plasma FSH has been presented by Vanmontfort et al. (1994). In consistence with the present findings, both studies by Vanmontfort et al. (1995) and Johnson et al. (1993) found a negative relationship between inhibin and FSH in female chicken that appeared to become functional at sexual maturity. In males, a possible negative correlationship between ir-inhibin and FSH was also observed at 135 days and thereafter. The negative feedback between ir-inhibin and FSH may be functionally established in males at the onset of puberty. In contrast, it has been shown in male chicken that this relationship was lacking at and shortly after puberty (Johnson and Brooks, 1996). Although Vanmontfort et al. (1995) did not find the negative correlation between inhibin and FSH according to their data in male chickens, they suggested that FSH in plasma would decrease further if more inhibin was present in males. In the present result, plasma concentration of ir-inhibin in male ducks was so high enough to suppress FSH secretion. A functional feedback between inhibin and FSH has been supposed to be exerted in the male rat prior to puberty but was lost by 60 days of age (Culler and Negro-Vilar, 1988). In male rats and monkeys, inhibin and FSH developed an inverse relationship during the pubertal process (Au et al., 1986; Marson et al., 1993). In male ducks, it is probably that intact bioactive inhibin secreted by testis subsequent to the establishment of testicular function plays a major role on regulation of FSH. Although there is no direct evidence supporting that testicular inhibin in avian species could regulate FSH secretion, initial studies about avian inhibin demonstrated that aqueous chicken testes preparations were effective in selectively reducing the postcastration rise in FSH in adult male rats (Bandivdekar et al., 1982, 1984).

The findings of no significant change in plasma LH of females agree with the reports of Johnson and Brooks, (1996). The higher plasma LH levels in males than in females were also observed in the present study, and the lower LH levels in females may have been a consequence of higher plasma estrogens, which are known to be more effective than other steroid in suppressing LH secretion (Sharp, 1975). In the present study, the results of testosterone in males and estradiol-17β in females are in consistence with the progressive increase in the steroidogenic processes in the testis and ovary during sexual maturation. These results agree with the results reported in chicken (Tanabe et al., 1979, 1981; Williams and Sharp, 1977).

It is widely agreed that the principal thyroidal hormone is T_4 and that the major source of T_3 is the peripheral deiodination of T_4 (Decuypere et al., 1979). Scanes et al. (1983) suggested that T_3 rather than T_4 exerted the major influence in the control of growth, particularly skeletal growth, in the fowl. Therefore, the decrease in plasma T_3 levels at middle ages seems to be associated with the relative low growth rate in the Shao duck. The interaction between the gonads and thyroid of birds is more pronounced than in mammals. The decrease of circulating T_4 levels from puberty onwards may be due to enhanced inhibitory effect of steroid hormones on thyroidal function (Bagchi et al., 1984; Sechman et al., 2000). Estrogen and progesterone have antithyroidal activity in juvenile ducks. In quail, plasma T_4 levels are depressed by testosterone treatment (Peczely, 1985).

In summary, these results demonstrated that the rise in inhibin is correlated with age of sexual maturity in the female while the rise in inhibin significantly precedes sexual maturity in the male. In addition, the progressive increase in steroid hormones (testosterone in males and estradiol-17β in females) may be consistent with the progressive increase in steroidogenic processes of the testis and ovary. Moreover, this study demonstrated that plasma concentrations of T3 and T4 followed the same time course in both sexes.

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