Endocrinological Changes before and after Removal of The Granulosa Theca Cell Tumor (GTCT) Affected Ovary in 6 Mares

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ABSTRACT. To clarify the endocrinological characteristics of the mares with granulosa theca cell tumor (GTCT), peripheral plasma samples from the 6 mares affected with GTCT were collected before and after the surgical removal of the affected ovary. Concentrations of testosterone (T), follicle stimulating hormone (FSH), luteinizing hormone (LH), immunoreactive-inhibin (ir-INH), progesterone (P) and estradiol-17 β (E₂) in the plasma samples were measured by radioimmunoassay. Before removal of GTCT in all cases, the concentrations of T were significantly higher than those of normal mares at the breeding and non-breeding seasons, whereas plasma concentrations of FSH, LH, ir-INH, P and E₂ were lower. After surgical removal of the affected ovary, the circulatory concentrations of T was declined, but the concentrations of other hormones were constantly low as compared with those of normal mares. The present study suggests that 1) the source of higher T may be due to the abnormal follicles in ovary of GTCT, 2) in the case of GTCT the elevated level of T is observed due to the lack of aromatase, and 3) the high level of T is a typical characteristics for GTCT in mares. It is also suggested 4) due to the elevated levels of T the concentrations of gonadotropins may be suppressed.

KEY WORDS: before and after removal of ovary, endocrinological finding, granulosa theca cell tumor (GTCT), mare.

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Granulosa Theca Cell Tumor (GTCT) are the most common ovarian tumor of the mare [2, 4, 13, 18]. GTCT occur unilaterally and contralateral ovary is usually small and firm [2, 4, 6, 7]. They arise from the sex cord stroma that lines the ovulation fossa [13, 14]. GTCT are usually benign and composed primarily of neoplastic granulosa cells but may also contain abnormal theca cells [18, 21, 22, 24]. GTCTs are usually steroid producing tumors, often associated with behavioral changes and poor reproductive performance. However, the surgical removal of the affected ovary is effective for the return of estrus. Therefore, it is very important to diagnose GTCT as early as possible for improvement of reproduction in mares.

The GTCT have been diagnosed by the ultrasonographic patterns [16, 26] and the measurements of sex steroid hormonal levels [19, 23, 24]. The stallion like behavior was associated with circulating testosterone (T) levels over 100 pg/ml [19, 24]. Endocrinological substances which may be produced by the tumor may be associated with behavioral changes and suppression of the hypothalamic or pituitary axis [12, 18, 23]. Suppression of pituitary gonadotropin output results in inactive condition of the contralateral ovary [1, 15, 16]. However, there are no reports about the source of high levels of T in GTCT affected mares.

In this paper to clarify the endocrinological characteristics of mares having GTCT, and to investigate the source of hormones, T levels were determined including follicle stimulating hormone (FSH), luteinizing hormone (LH), ir-inhibin (INH), progesterone (P) and estradiol-17 β (E₂)

before and after removal of the affected ovaries.

MATERIALS AND METHODS

Five mares of Thoroughbred and one mare of heavy breed were used in the present study. Diagnosis of GTCT was based on history, rectal palpation, transrectal ultrasound echographic image of ovaries and histopathological investigation. Rectal palpation revealed a large affected ovary with many follicular development and the unaffected contralateral ovary was usually small and inactive. On ultrasonographic scanning, multilobular "honeycomb" appearance was present. The histopathological diagnosis was done about the affected ovary after removal as shown in previous paper [11].

The size and weight of the affected ovary in 6 mares were given as follows; 13.0-9.7-6.5 cm (length-depth-width) and 530 g in case 1, 16.5-14.4-9.5 cm and 1,560 g in case 2, 18.0-13.5-11.0 cm and 2,000 g in case 3, 13.5-10.0-8.0 cm and 820 g in case 4 and 9.0-7.0-6.0 cm and 260 g in case 5 and 9.2-7.8-6.0 cm and 320 g in case No. 6. But the contralateral ovary was small and inactive in all cases.

Before and after removal of the affected ovary from these 6 mares, peripheral plasma samples were collected 2 days and 1 hr before operation and just before operation. After operation peripheral plasma samples were collected at 1, 2, 4, 6, 12 and 24 hr and 2, 3 and 7 days and were frozen under -20°C until the hormonal assay. As control, plasma samples from 18 Thoroughbred mares during the non-

Table 1. Concentrations of peripheral hormones before removal of GTCT in 6 mares

Case	T (pg/ml)	FSH (ng/ml)	LH (ng/ml)	ir-INH (pg/ml)	P (ng/ml)	E ₂ (pg/ml)
Case 1	224.01 ± 8.06	0.38 ± 0.04	0.17 ± 0.03	102.33 ± 27.91	0.51 ± 0.06	0.55 ± 0.31
Case 2	162.97 ± 12.62	0.21 ± 0.03	0.14 ± 0.03	186.22 ± 46.29	0.86 ± 0.18	5.27 ± 0.39
Case 3	488.42 ± 3.73	0.13 ± 0.01	0.14 ± 0.08	255.89 ± 63.94	0.28 ± 0.09	1.58 ± 0.45
Case 4	217.09 ± 35.79	1.21 ± 0.11	0.12 ± 0.01	157.37 ± 20.82	0.19 ± 0.05	0.72 ± 0.14
Case 5	360.74 ± 1.23	0.41 ± 0.04	0.13 ± 0.09	294.42 ± 22.69	0.11 ± 0.04	0.71 ± 0.14
Case 6	132.42 ± 6.84	0.41 ± 0.10	0.26 ± 0.04	156.56 ± 20.66	0.27 ± 0.09	0.42 ± 0.43
Mean ± SE	264.28 ± 55.09	0.46 ± 0.16	0.16 ± 0.02	199.25 ± 31.36	0.37 ± 0.11	2.45 ± 1.07

breeding season (October to December) and from 9 Thoroughbred mares during the breeding season (April to September) were also collected during in an anestrus condition and stored at same temperature until measurements of hormonal assay. Levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), immunoreactive (ir-) inhibin (INH), progesterone (P), testosterone (T) and estradiol-17 β (E₂) in peripheral blood were measured by specific radioimmunoassay (RIA) [9, 25, 26].

Plasma concentrations of FSH and LH were measured by using a rabbit antiserum against equine FSH (# AFP-2062096) provided by NIDDK, NIH, Bethesda, MD, U.S.A.) and LH (# AFP-240580). Highly purified equine FSH (# AFP-5022B) and LH (# AFP-5130A) were used for radioiodination and the reference standard. These materials were kindly provided by Dr. A.F. Parlow (National Hormone and Pituitary Program, Harbor-UCLA Medical Center, CA, U.S.A.). The concentrations of ir-INH in plasma in all cases were determined by using anti-bovine inhibin serum (TDNH-1) and bovine 32 KDa inhibin for radioiodination and the reference standard [9].

The concentrations of P, T and E₂ in plasma in all cases were determined as described previously [27] by using antisera to P (GDN#337), T (GDN#250) and E₂ (GDN#244). The inter and intra assay coefficient of variations of FSH, LH, ir-INH, P E₂ and T were 8.2% and 13.2%; 8.8% and 13.0%; 8.8% and 14.4%; 3.5% and 13.4%; 4.8% and 5.8% and 6.3% and 7.2%, respectively.

Most of the data were presented as mean $\pm$ standard error (SE). Significance of difference was compared by Student's unpaired *t*-test. All differences in values of $p < 0.05$ were considered to be significant.

RESULTS

Average concentrations of T, FSH, LH, ir-INH, P and E₂ before removal of GTCTs are shown in Table 1 and these changes before and after removal of GTCT in all cases are shown in Fig. 1 and Fig. 2.

Before surgical removal of GTCT in 6 mares, the average plasma concentrations of T were significantly higher (264.28 ± 55.09 pg/ml) ($p < 0.05$) as compared with those of normal mares at breeding (25.19 ± 3.01 pg/ml) and non-breeding seasons (46.94 ± 9.56 pg/ml). The average concentrations of FSH (0.46 ± 0.16 ng/ml), LH (0.16 ± 0.02 ng/

Fig. 1. Changes of plasma concentrations of FSH, LH, and ir-INH levels before and after removal of the affected ovary as compared with that of the normal mares.

ml), ir-INH (199.25 ± 31.36 pg/ml), P (0.37 ± 0.11 ng/ml) and E₂ (2.45 ± 1.07 pg/ml) were low as compared with those of normal mares at the breeding (3.3 ± 0.6 ng/ml in FSH, 6.81 ± 2.12 ng/ml in LH, 994.29 ± 128.67 pg/ml in ir-INH, 1.93 ± 0.67 ng/ml in P and 8.1 ± 1.56 pg/ml in E₂) and non-breeding seasons (3.6 ± 0.4 ng/ml in FSH, 1.32 ± 0.43 ng/ml

in LH, 516.21 ± 266.49 pg/ml in ir-INH, 1.3 ± 0.14 ng/ml in P and 5.08 ± 0.48 pg/ml in E₂).

After surgical removal of the affected ovary the average concentrations of T were abruptly declined in all cases

Fig. 2. Changes of plasma concentrations of T, P and E₂ before and after removal of the affected ovary as compared with that of the normal mares.

(166.92 ± 20.74 pg/ml) (Table 2) and were significantly lower ($p < 0.05$) as compared with those of before removal of the affected ovaries. The concentrations of FSH (0.52 ± 0.05 ng/ml), LH (0.30 ± 0.02 ng/ml), ir-INH (165.98 ± 35.47 pg/ml), P (0.61 ± 0.22 ng/ml) and E₂ (1.87 ± 0.54 pg/ml) (Table 2) were constantly low and there were no significant difference as compared with those of before and after removal of the affected ovary. The concentrations of T levels were significantly higher ($p < 0.05$) as compared with those of breeding and non-breeding seasons in normal mares.

Concentrations of T, FSH, LH, ir-INH, P and E₂ levels in the mares which were operated in breeding seasons (Case Nos. 1, 2, 4 and 5: Group A) were 241.20 ± 27.98 pg/ml, 0.49 ± 0.13 ng/ml, 0.14 ± 0.01 ng/ml, 185.08 ± 24.91 pg/ml, 0.41 ± 0.10 ng/ml and 1.86 ± 0.61 pg/ml, respectively (Table 3). The concentrations of T, FSH, LH, ir-INH, P and E₂ levels in the mares which were operated in non-breeding seasons (Case Nos. 3 and 6: Group B) were 186.48 ± 32.43 pg/ml and 130.78 ± 5.18 pg/ml; 0.46 ± 0.13 ng/ml and 0.63 ± 0.06 ng/ml; 0.32 ± 0.04 ng/ml and 0.28 ± 0.01 ng/ml; 238.22 ± 35.34 pg/ml and 216.60 ± 28.85 pg/ml, 0.35 ± 0.09 ng/ml and 0.41 ± 0.08 ng/ml; and 1.18 ± 0.30 pg/ml and 2.33 ± 0.29 , respectively (Table 3) and there were no significant difference between Group A and Group B.

DISCUSSION

The present study clearly demonstrates that an abundant of testosterone was secreted from GTCT in the present cases of mare, and were significantly higher as compared with the levels of normal mares at the breeding and non-breeding seasons. In addition, these high levels were abruptly declined by removal of GTCT. Until now, there are no reports about the source of testosterone in mares with GTCT. It is reported that aromatase play an important role to convert testosterone into estrogen in healthy large follicles [20]. It seems that in the case of GTCT the granulosa cells are unable to expressed aromatase, ultimately testosterone which are produce from the ovary cannot converted into estradiol, consequently the elevated levels of testosterone were observed in the case of GTCT in mares. It was reported that the GnRH secretion from the pituitary gland are inhibited by negative feedback mechanism of testosterone [5]. Therefore, subnormal levels of other hormones

Table 2. Concentrations of peripheral hormones after removal of GTCT in 6 mares

Cases	T (pg/ml)	FSH (ng/ml)	LH (ng/ml)	ir-INH (pg/ml)	P (ng/ml)	E ₂ (pg/ml)
Case 1	118.91 ± 15.00	0.40 ± 0.04	0.23 ± 0.03	88.60 ± 22.74	1.33 ± 0.20	2.18 ± 0.41
Case 2	131.10 ± 4.18	0.43 ± 0.11	0.36 ± 0.04	191.01 ± 19.87	1.28 ± 0.17	4.13 ± 0.60
Case 3	186.48 ± 32.43	0.46 ± 0.13	0.32 ± 0.04	238.22 ± 35.34	0.35 ± 0.09	1.18 ± 0.30
Case 4	181.16 ± 81.97	0.53 ± 0.17	0.32 ± 0.04	28.62 ± 62.57	0.16 ± 0.02	0.82 ± 0.28
Case 5	253.09 ± 56.57	0.67 ± 0.25	0.31 ± 0.04	232.85 ± 30.26	0.13 ± 0.04	0.58 ± 0.16
Case 6	130.78 ± 5.18	0.63 ± 0.06	0.28 ± 0.01	216.60 ± 28.85	0.41 ± 0.08	2.33 ± 0.29
Mean $\pm$ SE	166.92 ± 20.74	0.52 ± 0.05	0.30 ± 0.02	165.98 ± 35.47	0.61 ± 0.22	1.87 ± 0.54

Table 3. Concentrations of T, FSH, LH, ir-INH, P and E₂ levels in Group A and Group B

Group	T (pg/ml)	FSH (ng/ml)	LH (ng/ml)	ir-INH (pg/ml)	P (ng/ml)	E ₂ (pg/ml)
A						
Case Nos. (1, 2, 4 and 5)	241.20 ± 27.98	0.49 ± 0.13	0.14 ± 0.01	185.08 ± 24.91	0.41 ± 0.10	1.86 ± 0.61
B						
Case No. 3	186.48 ± 32.43	0.46 ± 0.13	0.32 ± 0.04	238.22 ± 35.34	0.35 ± 0.09	1.18 ± 0.30
Case No. 6	130.78 ± 5.18	0.63 ± 0.06	0.28 ± 0.01	216.60 ± 28.85	0.41 ± 0.08	2.33 ± 0.29

were observed.

It is reported in the mares with GTCT that the contralateral ovary is usually inactive condition [7, 8], but regains normal function following surgical removal of the affected ovary. It seems that after removal of the affected ovary the elevated levels of testosterone is declined and consequently FSH and LH can stimulate the contralateral atrophic ovary in inactive condition by the sufficient GnRH secretion, and the contralateral inactive ovary will regain its normal activity. Piquett *et al.* [20] suggested that the contralateral atrophied ovary will be caused by a lack of FSH stimulation, and GTCT may produce factors that inhibit gonadotropin secretion, although they did not measure the peripheral FSH levels in their mares with GTCT. In the cases with human granulosa cell tumor, it is shown to have contrary correlation between inhibin levels and FSH levels, namely higher levels of inhibin and lower levels of FSH in the peripheral circulation [5]. However, the present study indicates that plasma levels of FSH, LH, inhibin, progesterone and estradiol-17 β were lower. It seems that due to the negative feedback of testosterone to GnRH secretions, the lower levels of gonadotropins were observed in this study.

These results suggest that the elevated levels of testosterone were produced from the ovarian follicles in GTCT due to the lack of aromatase. The elevated levels of testosterone may suppress the gonadotropin secretion by negative feedback mechanism, and the high levels of testosterone is a good characteristics for GTCT in mares.

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