Clinical Findings before and after the Removal of Ovaries Affected with Granulosa Theca Cell Tumor (GTCT) in 16 Mares

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To clarify the clinical findings in mares with granulosa theca cell tumor (GTCT) and to determine reproductive activity after surgery, 16 mares with GTCT were studied. The GTCTs were found in 10.4 ± 0.9 year olds. The clinical signs manifested by the affected animals varied, and the mares showed abnormal behavior, that is, anestrus ($n=5$), continuous estrus ($n=8$) and stallion-like behavior ($n=3$), but most of the affected mares (11/16) were sterile for long periods (1–4 years), and in four animals GTCTs were detected at the post-partum period. In 11 of the 16 animals, the ovary containing tumor was enlarged on the right side, whereas the contralateral ovary was atrophied in all the 16 animals. Mares in which the affected ovary was surgically removed in the non-breeding season ($n=8$) had estrus 2.6 months earlier and became pregnant 2.9 months earlier than those operated on ($n=8$) in the breeding season, by resuming the reproductive function in the remaining ovary. All three mares that died from hemorrhage after the surgery were operated on in the breeding season. From these results we confirmed that GTCT is related to sterility for long periods in mares and that surgical removal of the affected ovary is effective in restoring reproductive function. And it is also suggested that the affected ovary should be removed in the non-breeding season.

Key words: clinical findings, granulosa theca cell tumor, mare, ovary, ultrasound

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Granulosa theca cell tumors (GTCT) are the most common ovarian neoplasms in the mare [11, 15, 26]. Usually they are benign, unilateral and composed primarily of neoplastic granulosa cells and abnormal theca cells [10, 15]. Suppression of pituitary gonadotropin output results in atrophy of the contralateral ovary [7], which is considered an important criterion for the diagnosis of GTCT [3, 12, 16, 26–28].

Because of these ovarian abnormalities, the affected mares become sterile for a long time although having abnormal sexual behavior [4, 6, 15], and surgical removal of the affected ovary is effective in restoring

estrus. Therefore, it is important to diagnose GTCT as soon as possible to improve reproductive performance in mares. GTCTs have been diagnosed by the ultrasonographic patterns [7, 28], and use of ultrasonography as an aid to the diagnosis of GTCT in the mare has been suggested [9, 20]. GTCT have been described as having a characteristic honeycomb-like appearance with distinct multilocular cysts on ultrasonography [8, 11, 15, 22, 26, 27]. It is important to study the common clinical findings of GTCT, because most papers about GTCT are limited to case reports.

This prospective study utilized a multidisciplinary approach, a) to describe the common clinical signs and diagnostic features associated with GTCT, and b) to gather information regarding reproductive activity after surgery.

Materials and Methods

A total of 15 Thoroughbred and one heavy breed with GTCT, which are considered to be representative of the cases seen in different parts of Japan from 1998 to 2001, were investigated in this study. The mares were presented for examination because of reproductive failure or changes in sexual behavior. GTCT was diagnosed by rectal palpation and ultrasonographic image of the ovaries. The affected ovaries were enlarged and the contralateral ovaries were small and atrophic on rectal palpation. After removal of the affected ovaries many large, medium and small follicles in ovaries were found in most of the cases and blood containing follicular fluid was also found in some follicles. On pathological examinations, the sections from the affected ovary were found to be composed of a thick granulosa cell layer with abundant cytoplasm in the theca externa cell layers and in the theca interna cell layers (unpublished data) as shown in another report [26]. At the time we also recorded which ovary was affected (i.e. left or right).

History information including breed, age, behavior, parity, period of sterility and days from parturition was used in this study. The decision whether the affected ovary should be removed was based on the abnormally large ovary, associated with infertility and behavioral changes. In the majority of animals (15/16) ovariectomy was induced under general anesthesia and maintained with an inhalation anesthesia isoflurane [Isoflurane; Dainippon Ltd.] via a cuffed endotracheal tube through a flank incision. Total anesthetic time varied between 40 and 100 min. In the other case it was performed under local anesthesia with 70 ml of 2% lidocaine hydrochloride [Xylocaine; Fujisawa Pharmaceutical Co., Ltd. Osaka] subcutaneously through a standing flank incision. Details of the surgical procedures were similar to those described earlier [15, 21] with minor modifications. Of the 16 mares, eight were operated on between October and December, which encompasses the non-breeding season of mares, four were operated on in April and another four were operated in July and September, which encompasses the breeding season of mares [9].

The excised ovaries (n=16) were measured and weighed, and another 20 ovaries from normal mares were examined as controls and compared with the GTCT ovaries. After the removal of the affected ovary, each mare was checked for the periods from surgery to estrous and to pregnancy.

Most of the data are presented as means $\pm$ standard error of the means (SEM). 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

The data on breed, age, estrous behavior, parity, period of sterility and days from parturition for each animal are shown in Table 1. The average age ($\pm$ SEM) of the mares affected with GTCT was 10.4 ($\pm$ 0.9) years. Seven of the 16 cases were over 13 years old. All the GTCT mares had abnormal sexual behavior. Of the 16 cases 5 (31.3%) affected mares were in the anestrus condition, 8 (50.0%) in continuous estrus and 3 (18.8%) showed signs of stallion-like behavior. Six primiparous and eight multiparous mares had a parity of 3.2 ± 0.8 (mean $\pm$ SEM) and the other 2 animals were maiden. Most of the 16 mares were sterile for more than one year, whereas four were at 10 to 180 days after parturition when they were found to have GTCT.

The surgical procedures and ovarian descriptions are summarized in Table 2. The incidence of GTCT in the right ovary (11/16, 68.8%) is higher than that in the left ovary (5/16, 31.2%). There were a number of follicles of various sizes containing blood follicular fluid (Fig. 1) in GTCT affected ovaries. The affected ovaries were spherical or lobular in appearance. In all cases the normal ovarian architecture was destroyed, the ovulation fossa was undetectable, and the tumor masses were multicystic on the cut surface (Fig. 2).

The 13 mares showed signs of estrus and became pregnant at 6.4 ± 0.4 months and 9.7 ± 1.5 months after surgery, respectively. Eight mares that were operated on in the breeding season (April to September) returned to the normal estrous cycle at 8.0 ± 0.6 months (n=5) and became pregnant at 12.0 ± 2.3 months (n=4) after surgery. On the other hand, the other eight mares operated on in the non-breeding season returned to the normal estrous cycle at 5.4 ± 0.2 months (n=8) and became pregnant at 9.1 ± 1.8 months (n=8) after surgery. The time from surgery to the return of the regular estrous cycle changed significantly ($p < 0.05$) but the time from surgery to pregnancy in the animals operated on in the breeding and the non-breeding season was not significantly different ($p > 0.05$).

The size and weight of the GTCT affected ovaries compared with those of normal ovaries are shown in Table 3. The weight of the neoplastic ovaries ranged

Table 1. Summary of the GTCT affected ovary

Cases	Breed	Age (Yrs)	Behavior**	Parity	Period of sterility (Yrs)	Days from parturition
1	Thoroughbred	13	Continuous estrus	4	3	*
2	Thoroughbred	7	Anestrus	1	0	10
3	Thoroughbred	8	Anestrus	3	0	180
4	Thoroughbred	6	Continuous estrus	1	0	90
5	Thoroughbred	5	Anestrus	1	0	49
6	Thoroughbred	13	Anestrus	1	2	*
7	Thoroughbred	10	Continuous estrus	2	3	*
8	Thoroughbred	15	Stallion like behavior	2	4	*
9	Heavy breed	5	Anestrus	0	0	*
10	Thoroughbred	13	Continuous estrus	1	2	*
11	Thoroughbred	14	Stallion like behavior	8	2	*
12	Thoroughbred	8	Continuous estrus	0	0	*
13	Thoroughbred	15	Continuous estrus	6	2	*
14	Thoroughbred	16	Stallion like behavior	10	1	*
15	Thoroughbred	10	Continuous estrus	4	1	*
16	Thoroughbred	8	Continuous estrus	1	1	*
mean $\pm$ SE		10.4 $\pm$ 0.9		3.2 $\pm$ 0.8***	2.1 $\pm$ 0.3	

All cases were diagnosed as GTCT in the breeding season. *: maiden in cases 9 and 12, or sterile for more than 1 year (1–4 years). **: Anestrus: 5 cases; Continuous estrus: 8 cases; Stallion-like behavior: 3 cases. ***: mean $\pm$ SEM in 14 multiparous mares.

Fig. 1. Ultrasonographic image of the contralateral ovary (Left) and the GTCT affected ovary (Right) from case No. 9. Note the enlarged ovary with a multilocular cyst (F) and some bloody follicular fluid (BF) in the GTCT affected ovary and the atrophic and inactive contralateral ovary.

Fig. 2. Cut surface of the GTCT ovary from case No. 2. The multilocular cyst had a characteristic honeycomb appearance on ultrasonographic examination.

from 80 g to 2,000 g, and the average weight was approximately 6 times heavier than that of normal ovaries. The size and weight of the affected ovary

associated with ovariectomy performed in the breeding and non-breeding season were (11.1 ± 1.4 cm in length, 9.5 ± 1.0 cm in depth, 7.3 ± 0.9 cm in width and 12.1 ± 1.9 cm in length, 9.8 ± 1.5 cm in depth, 7.7 ± 0.9 cm in width) and (570.8 ± 167.2 g and 753.4 ± 269.3 g), respectively. The weight of the affected ovaries that were operated on in the non-breeding season was not significantly different ($p > 0.05$) from those in the breeding season.

Table 2. Clinical findings in mares with GTCT

Case	affected ovary	surgery	Season of operation		Ovarian Characteristics	Follow-up after surgery
			breeding	non-breeding		
1	Right	FI*	April		13.0–9.7–6.5 cm 530 g	Died
2	Right	FI	April		16.5–14.4–9.5 cm 1560 g	Estrus; 7 months Pregnant; 13 months
3	Left	FI	April		13.5–10.0–8.0 cm 820 g	Estrus; 10 months Pregnant; unknown
4	Right	FI	July		9.0–7.0–6.0 cm 260 g	Estrus; 7 months Pregnant; 7 months
5	Right	FI	April		15.0–12.0–12.0 cm 710 g	Died
6	Left	FI	September		7.0–10.0–7.5 cm 410 g	Estrus; 9 months Pregnant; 9 months
7	Right	FI	September		8.5–7.5–5.5 cm 196 g	Estrus; 7 months Pregnant; 18 months
8	Left	FI	July		6.5–5.5–3.5 cm 80 g	Died
9	Right	SFI**		December	18.0–13.5–11.0 cm 2000 g	Estrus; 5 months Pregnant; 8 months
10	Right	FI		November	9.5–9.0–6.5 cm 380 g	Estrus; 5 months Pregnant; 5 months
11	Left	FI		October	9.5–7.5–8.0 cm 400 g	Estrus; 6 months Pregnant; 17 months
12	Left	FI		October	6.0–5.0–4.2 cm 150 g	Estrus; 6 months Pregnant; 7 months
13	Right	FI		October	8.0–7.5–6.5 cm 214 g	Estrus; 6 months Pregnant; 7 months
14	Right	FI		November	20.0–17.0–10.0 cm 1500 g	Estrus; 5 months Pregnant; 16 months
15	Right	FI		December	13.5–9.0–7.5 cm 630 g	Estrus; 5 months Pregnant; 6 months
16	Right	FI		November	unknown	Estrus; 5 months Pregnant; 7 months
5 11			8	8		Estrus: 6.4 ± 0.4 months Pregnant: 9.7 ± 1.5 months

*FI; Flank incision; **SFI; Standing Flank incision.

Discussion

Granulosa theca cell tumors (GTCTs) are by far the most common ovarian tumors in the mare and they are macroscopically characterized by their large size and multiloculated cystic architecture [24, 26]. Although a number of papers on GTCT in mares have been published, most of them were limited to case reports, and therefore little is known about common and /or specific clinical findings in GTCT mares.

In this study, we observed a large and characteristic honeycomb appearance in the GTCT ovary in all the cases as in other reports [10, 17].

In our study, GTCTs were unilateral and benign, and the contralateral ovary was atrophic in all the cases, as described by others [3, 4, 15–18, 21, 23]. It is clear that these findings are considered an important criterion for the diagnosis of GTCT. Piquette *et al.* [22] have demonstrated that the equine GTCT express the mRNA for inhibin and also secrete inhibin subunits, though they did not measure the peripheral inhibin and FSH levels in their mares with GTCT. It has also been reported that GTCT affected mares secrete abnormal levels of progesterone, testosterone and estradiol [12, 17]. Therefore, in future it will be necessary to study whether or not abnormal hormonal levels may be responsible for the atrophy and inactivity

Table 3. Size and weight of the GTCT affected ovaries as compared with those of the normal ovaries

Cases	Size (cm)	Weight (g)
GTCT (n=16)	6.0–20.0 × 5.0–17.0 × 3.5–12.0 11.5 ± 1.4 × 9.6 ± 0.9 × 7.5 ± 0.6 (length) (depth) (width)	80–2000 656.0 ± 150.2
normal (n=20)	5.5 × 3.5 × 3.0 (minimum) (length) (depth) (width) ↓ 9.0 × 6.0 × 6.0 (length) (depth) (width) (maximum)	35.8–193.4 84.3 ± 8.0

a) in the case of operation in breeding season (n=8); weight 80–1560 g (570.75 ± 167.19), size (11.1 ± 1.4, 9.5 ± 1.0, 7.3 ± 0.9 cm). b) in the case of operation in non-breeding season (n=8); weight 150–2000 g (753.43 ± 269.30), size (12.1 ± 1.9, 9.8 ± 1.4, 7.7 ± 0.8 cm). The size and weight of the affected ovaries in breeding season are not significantly different ($p>0.1$) compared with those in non-breeding season.

observed in the contralateral ovary.

In most of our cases, GTCT was found in sterility for long period, but in some cases it was found soon after parturition. Pierson and Ginther [21] suggest that the tumors develop during pregnancy but do not interfere with gestation or parturition. In the mare the extensive dissemination of GTCT suggests that it was present during gestation, and that parturition was normal without surgical removal of the affected ovary [13]. The endocrine changes during pregnancy are peculiar to the mare among domestic animal species because of the development of the temporary hormone producing structure, the endometrial cups, from which equine chorionic gonadotropin (eCG) is secreted. In man, human chorionic gonadotropin (hCG) is a marker for ovarian tumor [2]. An increase in hCG occurs in breast, gastro-intestinal tract, lung, melanoma and ovarian cancer. hCG is also increased in some non-cancerous conditions such as normal pregnancy, cirrhosis, duodenal ulcer and inflammatory bowel disease. Although eCG is functionally different from hCG and GTCTs are pathologically different from human ovarian cancer, it seems that the increase in eCG in mares may lead to GTCT during pregnancy.

In the present study GTCT affected mares showed signs of anestrus (5/16, 31.3%), continuous estrus (8/16, 50.0%) and stallion-like behavior (3/16, 18.8%). This result is different from the previous report [4] in which GTCT affected mares had anestrus (5/14, 35.7%), continuous estrus (1/14, 7.1%) and stallion

like behavior (8/14, 57.1%). Our results are also different from those obtained by others [17] in which affected mares had anestrus (20/63, 32%), continuous estrus (14/63, 22%) and stallion-like behavior (29/63, 46%). Behavioral changes may be associated with abnormal hormonal production by the tumor [14, 17]. In cattle [1] the tumor cells presumably secrete estrogen in the early stages of development of GTCT, and this may cause continuous estrus in the affected animals. Most of the tumor tissue then undergoes luteinization that may lead to an anestrus condition, and may result in virilism in long standing cases [1]. If the estrous behavior in GTCT mares changes with time in the same manner as in GTCT cows, the tumor may have been at a relatively early or mid stage of development of the condition when many of our cases were diagnosed as GTCT.

The incidence of GTCT in the right ovary (64.7%) was higher than that in the left ovary (35.3%) in this study but the number of GTCT cases that affected the right ovary is about the same as the number that affected the left ovary as reported previously in various different breeds including the Thoroughbred [4–7, 10, 11, 19, 22, 26, 28]. Therefore, the incidence of GTCT is probably not related to the side on which the ovary is located.

Surgical removal of the affected ovary in our cases was performed both in the breeding and in the non-breeding season. The period between the operation and the resumption of the normal estrous cycle when

the surgery was performed in the non-breeding season was shorter than that in the breeding season. Mares that are operated on late in the breeding season may not return to cyclic ovarian activity until the next spring [25]. In the present study, four mares were operated on April because the veterinarian expected that the operated mare would have a normal estrous cycle in the same breeding season. Nevertheless, no mare operated on in April or at other times in breeding season returned to the normal estrous cycle in the same breeding season except one (case No.2). In addition, the operation in the breeding season seemed to have a higher degree of risk than that in the non-breeding season because in the present study all of the three mares that died from hemorrhage after surgery were operated on in the breeding season. Presumably the ovarian artery and vein become larger in the breeding season because of higher levels of hormonal activity than in the non-breeding season. Therefore, it may be more complicated to properly ligate ovarian blood vessels in the breeding season. Post surgical complications occurred in most of the mares and some mares died after surgery [17].

The size and weight of the affected ovaries that were operated on in the non-breeding season were not significantly different from those in the breeding season.

GTCT is considered to produce abnormal levels of different kinds of hormones [26] and this might be related to the clinical signs and the differentiation of ovarian tumors. Hormonal profiles for the 16 mares in this study and their relationships to the clinical signs manifested by each animal will be the subject of a future report to confirm the final diagnosis and obtain a more precise grasp of the clinical findings.

In conclusion, although the majority of the mares with GTCT were sterile for a long time, some had the tumor in the postpartum period. And it is clear that surgical removal of the affected ovary is effective in restoring reproductive function in the remnant contralateral atrophic ovary. Also it is recommendable that the GTCT should be removed in the non-breeding season because the operation is more effective in the non-breeding season than that in the breeding season.

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