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Granulosa cell Tumour (GCT) associated with Pyometra in a golden retriever bitch

Priyanka Narwade, B Balamurugan, Rabindra Mohan Mishra, Priya Ranjan Kumar, Vinod Kumar, P Suvaneeth, Dayanidhi Jena and Sanjay Kumar Ravi

Abstract
A 6 years old nulliparous Golden Retriever was presented to the Teaching Veterinary Clinical Complex, FVAS, RGSC-BHU Mirzapur, with a history of sanguineous vaginal discharge with vulvar oedema for two months. It was previously treated for pyometra, but no improvement was observed. Ultrasonographical examination revealed multiple anechoic structures in the ovary. The ovarian mass was excised after separation from the ovarian bursa and extraovarian tissues while performing ovariohysterectomy and subjected to histopathological examination. The animal recovered uneventfully after treatment. On histopathological examination, the tumour tissue appeared like a bunch of follicles in a lobular pattern separated by connective tissue stroma with typical Call Exner bodies, indicative of a granulosa cell tumor.

Keywords: Bitch, pyometra, granulosa cell tumour, ovariohysterectomy

Introduction
Ovarian tumors are uncommon in the bitches, accounting for approximately 1% of all neoplasms. There is an increased incidence of ovarian neoplasia in older bitches; the mean age of occurrence is 8 years (Withrow and Susaneck 1986) [18]. The most important are granulosa cell tumors, which may become very large and produce clinical signs related to the mass effect or ascites. They do not frequently metastasis and are usually endocrinologically inactive; however, they may secrete: progesterone, resulting in the absence of cyclicity, cystic endometrial hyperplasia, and pyometra; or estrogen, resulting in signs of persistent oestrus or possibly bone marrow suppression. Rarely, alopecia is a presenting clinical sign (Noakes *et al.*, 2019) [9]. Many synonyms are defined for granulosa cell tumors, including feminizing Mesenchymoma, gynoblastoma, folliculoma, basal cell carcinoma, follicular adenoma, or follicular epithelioma (Sivacolundhu *et al.*, 2001) [15]. Sex cords, or primitive cortical lobules, and the specialized stroma or mesenchyme of the developing gonad can potentially originate from a granulosa cell tumor of the ovary (Pichon *et al.*, 2011; Klopfleisch *et al.*, 2016) [12, 14]. The incidences are high, especially in intact older bitches. It is usually unilateral, reaching up to 20–30 cm in diameter. Granulosa cell tumors of the ovary originate from granulosa cells of ovarian follicles and can present solid or cystic follicular patterns (Maya *et al.*, 2017; Matos *et al.*, 2019) [6, 5]. Granulosa cell tumors can exert hormonal activity resulting in estrogen (Buijtels *et al.*, 2010) [2], anti-Mullerian hormone, and alpha-inhibin secretion (Marino and Zanghi, 2013) [7]. The main clinical signs of granulosa cell tumors include irregular estrous, serosanguineous vaginal discharge, vaginal hyperplasia, ovarian cyst, pyometra, vaginal prolapse, and dermatological lesions such as symmetric alopecia and hyperpigmentation (Marin *et al.*, 2014) [8]. Due to very discrete clinical signs, the diagnosis of ovarian tumors in bitches is usually complicated, as it is occasionally confused with other conditions. In the present case, the tumor was diagnosed by histopathological examination after ovariohysterectomy.

Case History and Observations

A 6 years old nulliparous Golden Retriever was presented to the Teaching Veterinary Clinical Complex, Faculty of Veterinary and Animal Sciences, BHU-Mirzapur, with a history of sanguineous vaginal discharge and vulvar edema for the last 2 months and a normal appetite. The bitch was active and alert, and all the vital parameters were within the normal range. The bitch was treated for pyometra for 20 days, but there was no improvement. Ultrasonographical examination was showed enlarged anechoic multiple, nodular structures on the ovary. After diagnosis it was decided to go for an ovariohysterectomy. The liver function test and kidney function test were performed, and the following values were observed.

Table 1: Findings of liver and kidney function test

Liver function test	Observed Values
Albumin	2.35gm %
Total proteins	4.59gm %
Indirect Bilirubin	0.03
Direct Bilirubin	0.06
SGOT/AST	12.7 IU/L
SGPT/ALT	53.74IU/L
LDH	76 U/L
Alkaline Phosphatase	28 L
Kidney function test	
Blood urea	23.5 mg %
Blood Urea nitrogen	10.98 mg %
Serum creatinine	0.5mg %

Treatment and Discussion

The bitch was subjected to 12 hours of fasting. The surgical site was prepared aseptically. Premedication was done with atropine @ 0.04 mg/kg b.wt. S/C, followed by xylazine @ 1 mg/kg b.wt. I/M, and butorphanol @ 0.4 mg/kg b.wt. I/M. Diazepam (0.5 mg/kg b.wt.) I/V for induction and maintained with 3% isoflurane. The animal was positioned in dorsal recumbency. The midline incision was made cranially, one-third from the umbilicus. The stab incision was placed on the linea Alba after the elevation of the rectus muscle. Abdominal and peritoneal muscles were separated, and the uterus was exposed. After exteriorization of the right ovary, it was found to have enlarged; severe adhesions and a large mass on the ovary with multiple growths at about 11×6 cm. The mass was excised, and representative samples were taken from different parts of the tumor mass and subjected to routine histopathological examination (Suvaneeth *et al.*, 2015) [16]. An ovariohysterectomy was performed to prevent further complications. Tran fixation and ligation of the ovarian arterio-venous below the ovary using absorbable suture material at the cut end of the pedicle. The pedicle was grasped and inspected for bleeding. The same procedure was repeated on the left horn of the uterus. The uterus and ovaries were removed after ligation, and Tran's fixation was done using cat gut no. 1. Abdominal incisions were closed with a simple interrupted suture pattern using synthetic absorbable suture material, like vicious subcutaneous tissue and skin, which were closed routinely with a subcuticular suture pattern and an interrupted suture, respectively. The bitch was treated postoperatively with inj. Taxim 60 mg/kg b. wt I/V, inj. DNS 500 ml I/V, inj. RL 500 ml I/V, inj. Botropase 2 ml I/M, and inj. Mucomix 70 mg/kg I/V for 5 days. The bitch was recovered uneventfully.

Macroscopically, the right ovarian tumor was 11x6 cm, smooth to the touch, and had irregular cysts with a firm consistency. The cut surface was mostly solid, with some cystic follicles and hemorrhage in some areas. Microscopically, the tissue appeared like groups of follicles in a lobular pattern separated by connective tissue stroma. The cells had foamy cytoplasm and vesicular nuclei and were found in solid sheets with loosely distributed eosinophilic amorphous content in between. Several mitotic figures were also observed. Radial palisade arrangements of two or more rows of vesicular-type cells with condensed chromatin and multiple nucleoli were seen around eosinophilic, proteinaceous material in some of the lobules, indicating Call Exner Bodies typical of granulosa cell tumor. Based on the gross and histopathological characterization, the tumor was identified as a granulosa cell tumor.

The present condition in a golden retriever bitch showed the possible relationship between granulosa cell tumor and pyometra. Granulosa cell tumor is more common in the left ovary of middle- to older-aged bitches (Saforna *et al.*, 2003) [14], although here the incidence of ovarian tumors was observed on the right side, unilateral, and there is continuous sanguineous vaginal discharge from 2 months due to the increased estrogen level in the blood. Granulosa cell tumor may produce and increase the secretion of hormones such as estradiol, progesterone, and inhibin (Pluhar *et al.*, 1995) [13]. As a result of its hormonal secretion, granulosa cell tumor often induces persistent estrus, vulvar swelling with discharge, and alopesia (Buijtels *et al.*, 2010) [2]. Granulosa cell tumor predispose to cystic endometrial hyperplasia and pyometra. Hormonal disturbances have a major role in cystic uterine atresia and cystic endometrial hyperplasia. The secreted progesterone can enhance endometrial growth and develop cystic endometrial hyperplasia and cystic uterine atresia. In the present, bitch cystic endometrial hyperplasia was observed. Suggesting that it is associated with increased progesterone in the blood. About 80% of granulosa cell tumor is benign. An ovariohysterectomy was performed on the basis of an ultrasonographic examination. Radiographic and ultrasonographic examinations could benefit the diagnosis. Macroscopic examination of the right ovary revealed large cysts filled with brown, clear, and jelly materials. The gross appearance of the uterine endometrium evidenced that it was filled with many cysts. They had different sizes, and some of them were filled with clear fluid. Histopathology revealed a follicular pattern of the tumor, separated by a moderately thick fibrous stroma, which is not uncommon for granulosa cell tumors. There was eosinophilic, amorphous material found throughout the irregularly arranged sheets of neoplastic cells. Neoplastic cells are uniformly small, round to ovoid-shaped, with the nuclei round or ovoid with scattered chromatin and prominent nucleoli. The cytoplasm of these cells was scanty, granular, and slightly eosinophilic. The observations were similar to those of Patnaik and Greenlee (1987) [11], Matos *et al*. (2019) [5], and Oviedo-Penata *et al*. (2020) [10]. Granulosa cell tumor is composed of cells similar to developing follicles. Cells are round with small hyperchromatic nuclei of variable sizes, and mitoses are numerous. Some cells are similar to sertoli cells, with foamy and poorly colored cytoplasm (Balamurugan and Vipin Maurya 2022) [1].

Fig 1: Ultrasonographical examination showed multiple anechoic cystic structures in the ovary

Fig 2: Exposed ovarian tumor mass

Fig 3: Enlarged ovarian mass with cyst

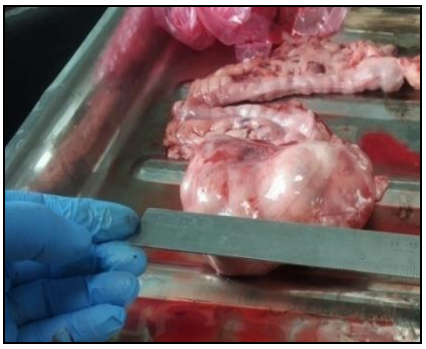

Fig 4: Excised ovarian tumor mass

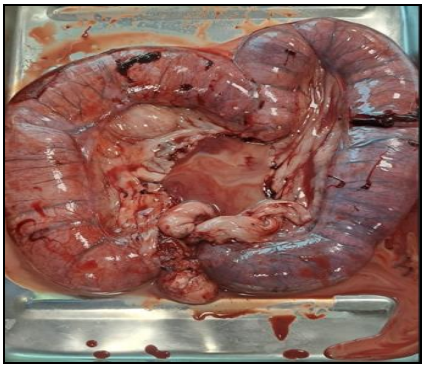

Fig 5: Accumulation of pus within the uterus

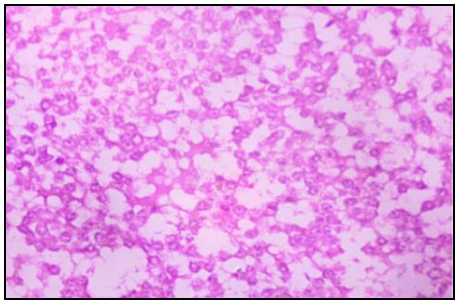

Fig 6: Neoplastic cells found in solid sheets with amorphous eosinophilic content (H&E x 400).

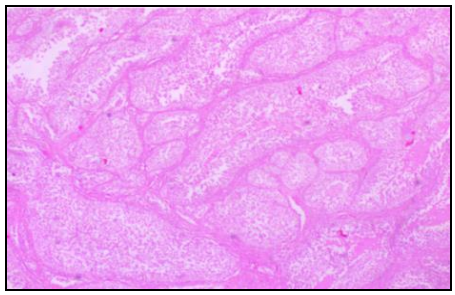

Fig 7: Lobular pattern of the tumour separated by fibrous tissue Stroma. (H&E x 40).

Conclusion

Granulosa cell tumor is rarely found in bitches and this condition can be diagnosed on the basis of clinical signs, ultrasonographic examinations, and histopathological findings. Generally, ovarian hysterectomy is the choice of surgical correction for this condition.

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