



# Ovarian Granulosa Cell Tumor and Complex Mammary Adenocarcinoma in a Bitch

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## Abstract

An 11-year old boxer bitch was presented to the Department of Obstetrics and Gynaecology clinic for routine ovariohysterectomy and coincidentally an ovarian mass was detected which was diagnosed as granulosa cell tumor (GCT) in pathologic examinations. Blood sample was collected for hormonal analysis during this surgery in which estrogen concentration was under the reference range, progesterone concentration was normal. Mammary gland enlargements were also detected on the dog during initial examination with no other sign regarding to vulvar discharge/swelling. One month later following ovariohysterectomy, the dog was presented again for bilateral total mastectomy. Tumoral tissues were diagnosed as complex adenocarcinoma grade II, histopathologically. The possible interaction and promotional effect of GCT on the development of mammary tumors (MTs) is not yet fully understood. Regarding to serum estrogen and progesterone concentrations were not above the reference ranges suggest us GCT did not secrete hormones yet. In conclusion, this case is important as it reports the possible effect of GCT on the development of MTs and suggests not to ignore this disease in a dog with mammary tumours.

## Özet

### Bir Köpekte Ovarian Granüloza Hücre Tümörü ve Kompleks Meme Adenokarsinomu

On bir yaşlı boxer köpek rutin kısırlaştırma için Doğum ve Jinekoloji Anabilim Dalı kliniğine getirildi ve histopatolojik olarak granüloza hücre tümörü (GHT) olarak teşhis edilen bir kitle tesadüfen saptandı. Ameliyat esnasında hormonal analiz için kan örneği toplandı ve bu kan örneğinde östrojen konsantrasyonu referans değerlerin altında, progesteron konsantrasyonu ise normal olarak tespit edildi. Köpeğin ilk muayenesi esnasında meme bezlerinde genişlemeler tespit edildi ve herhangi bir vulvar akıntı/şişlik tespit edilmedi. Kısırlaştırmayı takiben bir ay sonra köpek total bilateral mastektomi için kliniğimize tekrar getirildi. Histopatolojik olarak tümörel kitleler kompleks adenokarsinom tip II olarak tespit edildi. Köpekte hem GHT hem de meme tümörü aynı anda bulunduğu halde, GHT'nün köpek meme tümörü gelişimi üzerine etkisi tam anlamıyla anlaşılamadı. Serum östrojen ve progesteron konsantrasyonlarının referans değerlerden yüksek olmamasına bağlı olarak, GHT'nün henüz hormon salgılamadığı düşünüldü. Sonuç olarak, bu olgu GHT'nün köpek meme tümörünün gelişimi üzerine olası etkisinden bahsetmesinden ve köpekte meme tümörü varlığında bu hastalığın göz ardı edilmemesi gerekliliğini önermesinden dolayı önemlidir.

## Introduction

Canine ovarian tumours are categorized into three groups as follows; germ cell tumours, sex-cord stromal tumours and epithelial cell tumours (Kennedy et al., 1998). Granulosa cell tumours (GCT) arise from sex cord stromal cells in canine ovary (Tavasoli and Solati, 2011) and have been usually reported in bilateral localization in dogs (Kennedy and Miller, 1993). Granulosa cell tumours can produce hormones thus increase estradiol,

progesterone and a-inhibin concentrations (Pluhar et al., 1995). The progesterone secretion by the GCTs leads to endometrial growth and glandular secretion that cause cystic-endometrial hyperplasia-pyometra complex (Feldman and Nelson, 1987). As a result of this hormonal secretion, the dog experiences persistent estrus, vulvar swelling, and alopecia (Johnston et al., 2001). Besides, in most cases of GCTs serosanguineous vulvar discharge is frequently noticed in the bitches (Feldman and Nelson, 1987).

Mammary tumours (MTs) are the most common neoplasms in intact female dogs (Sleeckx et al., 2011). Different factors such as age, breed, genetic predisposition, hormones and growth factors, cyclooxygenase-2 expression, and diet may act on the formation of MTs (Lavalle et al., 2009; Rivera et al., 2009). During the long luteal phase of the oestrous cycle of the bitch, the mammary glands expose to a high concentration of progesterone (Schaefer-Okkens et al., 2005) which induces the lobulo-alveolar development with hyperplasia of gland epithelia and myoepithelial cells (Rutteman, 1990). Also, oestrogens promote the ductal growth of the mammary gland in the bitch (Rutteman, 1990). Ovarian steroids stimulate the growth of normal mammary tissue under physiological conditions and thus their proliferative effect on epithelium may lead the formation of neoplastic feature (Genuth, 1998; Sorenmo et al., 2000). This occurs at every oestrus cycle and makes the bitch to be prone to MTs (Rutteman, 1990).

Progesterone and estrogen receptors were detected in both normal and neoplastic tissues previously besides serum steroid hormone levels of bitches with malignant MTs were found significantly higher when compared to healthy bitches with benign MTs (Gerald et al., 2000; de las Mulas et al., 2005).

**Table 1.** The result of haematological parameters before the surgery.

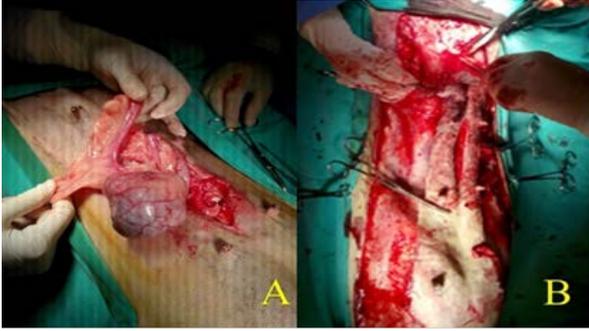
**Tablo 1.** Ameliyat öncesi hematoloji parametrelerinin sonuçları.

Test	Before Operation	Reference Range
RBC ( $\times 10^6/\mu\text{L}$ )	7.04	5.5-8.5
HGB (g/dL)	16.4	12-18
HCT (%)	52	37-55
WBC ( $\times 10^3$ )	9.5	6-17
PLT ( $\times 10^3 \mu\text{L}$ )	507	200-500
MCV	73	60-77
MCH (pg)	23	19.5-26
MCHC (%)	32	32-36
Glucose (mg/dL)	102	60-125
BUN (mg/dL)	34	7-27
Creatinine (mg/dL)	0.9	0.4-1.8
AST (IU/L)	37	5-55

Although GCT and mammary neoplasia are present in this bitch at the same time, the effect of GCT on the development of canine mammary tumour is not fully understood. Serum estrogen and progesterone concentrations were not above the reference ranges suggest us GCT did not secrete hormones yet.

## Case

An 11-year old boxer bitch was presented to the Department of Obstetrics and Gynaecology clinic for the request of the client for routine ovariohysterectomy. During initial examination mammary gland enlargements were detected as multiple nodules present at the inguinal and thoracic mammary glands. Before the surgery, complete blood cell count and biochemistry were performed to the dog (Table 1). Total bilateral mastectomy was recommended to the client a month after ovariohysterectomy. The dog underwent ovariohysterectomy under general anesthesia. To maintain anesthesia propofol (Pofol ampul®, Dongkook Pharm, Korea) at 6 mg/kg dose iv and 3-4 % isoflurane (Foran liquid®, Abbott Laboratories, England) and O<sub>2</sub> combination were used. Median line was preferred for the operation. As it was assumed as a routine ovariohysterectomy, any blood sample was not collected for hormonal analysis. But while a mass was detected (Figure 1A), another blood sample was collected immediately during the surgery and centrifuged 3000 rpm. for 10 minutes. Serum was discarded and stored at -20°C till estradiol and progesterone analysis (Vetlab, Immulite® 2000 XPI Immunoassay system, Siemens) (Table 2). After the surgery, the tissues were sent to Pathology Department for histopathology. In the macroscopical examination, the tissue was 10x1x1 cm in diameter. One of the ovary was 5x4.5x3.5 cm in diameter and cystic. It had a necrotic content and solid proliferations. Microscopically other ovary and uterus were appeared normal. The samples were fixed in 10 % neutral buffered formalin, routinely processed, and embedded in paraffin. Five-micrometer sections were mounted on glass slides and stained with hematoxylin and eosin. Microscopically proliferative round, abundant, vesicular nuclei epithelial cells with trabecular formation were observed in big ovary including Call-Exner bodies macrofollicular, trabecular, solid and insular patterns (Figure 2-3), cuboidal to polygonal cells in various patterns (Figure 3), in some section nuclear pleomorphism in those cells was detected. Necrosis was also evident in this area. Mitotic figures were present in the some anaplastic tumoral cells (Figure 4). According to the histological evaluation, granulosa cell tumor in ovary was diagnosed. Taking the severely differentiated atypical cells making mitosis and necrotic areas in some parts of the neoplastic tissue, it was evaluated as malign. In the ovarian tissue, follicular cysts with various sizes were also detected. Besides, endometrial hyperplasia and cystic changes were noticed.



**Figure 1.** The determined mass during routine ovariohysterectomy (A), Performing total bilateral mastectomy on the bitch (B).

**Şekil 1.** Rutin kısırlaştırma esnasında tespit edilen kitle (A), Dişi köpekte total bilateral mastektominin uygulanması (B).

A month later, the dog was presented to our clinic for bilateral total mastectomy (Figure 1B). Complex adenocarcinoma grade II was diagnosed in pathological examination of these tissues.

**Table 2.** Estradiol and progesterone concentrations after hormonal analysis.

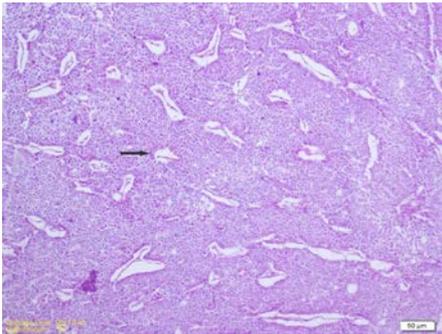
**Tablo 2.** Hormonal analiz sonrası östradiol ve progesteron konsantrasyonları.

Test	Serum Values	Reference range
Estradiol	9.0 pg/mL*	10-90 pg/mL*
Progesterone	4.0 ng/mL**	0-5 ng/mL**

\*: pg/mL: picogram/milliliter

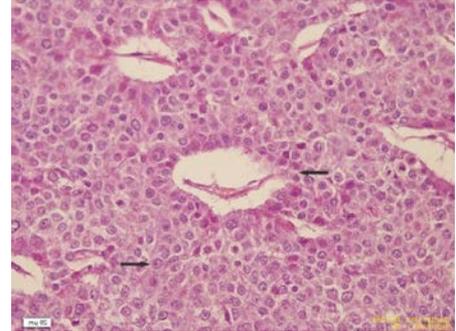
\*\* : ng/mL: nanogram/milliliter

During postoperative periods ceftriaxone sodium (Novocéf flc.®) intramuscular (im) at a 20 mg/kg dose for a week, and vitamin B and C complex (Hepargrizeovin amp.®), 1 mg/kg im Ranitidin (Ulcuran amp.®), 2.2 mg/kg Carprofen (Rimadyl tablet®) were administered. An elizabethan collar was placed in order to prevent the dog from accessing the sutures.



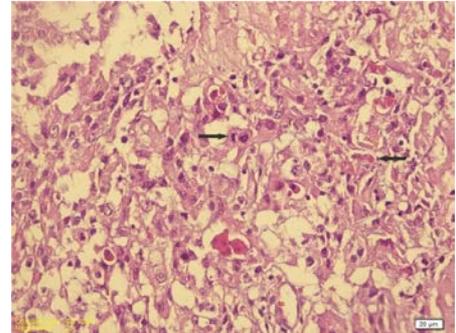
**Figure 2.** Call-Exner bodie (right arrow).

**Şekil 2.** Call-Exner cisimciği (sağ ok).



**Figure 3.** Macrofollicular, trabecular, solid (right arrow) and insular patterns (left arrow).

**Şekil 3.** Katı, trabeküler, makrofoliküler (sağ ok) ve adacıklı görünüm (sol ok).



**Figure 4.** Mitose (right arrow), necrosis (left arrow).

**Şekil 4.** Mitoz (sağ ok), nekroz (sol ok).

### Discussion and Conclusion

Granulosa cell tumors occur frequently in middle aged or old dogs (Klein, 2001) compatible with the age of 11-year old in the current report which was diagnosed during the surgery coincidentally. The great majority of the previous scientific reports advise the usage of ultrasonography in the diagnosis of GCT (Diez-Bru et al., 1998). As we observed this case arbitrarily we couldn't have the possibility to perform ultrasonographic examination before surgery.

In most of the reports about GCTs, the presence of serosanguinous vulvar discharge was highlighted (Pluhar et al., 1995; Feldman and Nelson, 1987). According to our examination and anamnesis given by the owner there was no vulvar discharge from the dog regarding pus accumulation in the uterus related to GCT formation in the ovary. As GCTs can produce estrogen and progesterone, persistent estrus or proestrus, vulvar swelling, vaginal discharge, vaginal cornification, swollen mammary glands, pyometra, symmetrical alopecia, behavioral changes, and estrogen myelotoxicity can be seen (Ball et al., 2010). Spoor et al. (2014) reported a

markedly enlarged mammary glands and that they attributed this mammary enlargement could be related with the GCT organised from ovary and its estrogen secretion. In this text we can not report like that kind of findings as our hormonal profile is between normal ranges.

A higher percentage of canine GCTs have been reported malign which metastasize to regional lymph nodes and organs (Tavasoli and Solati, 2011). But nonfunctional GCTs have been reported previously that have usually no clinical signs related to reproductive tract (Zanghi et al., 2007). Although the tumor in the present case diagnosed as malignant, we believe that the bitch did not experience any clinical symptoms related to steroid hormone presence due to its lowrise hormonal profile or due to the dissection of ovaries in the early development stage of the tumour. Moreover, Tunca et al. (2011) reported GCT in two bitches that they did not encounter any sexual behavior variations, too.

In conclusion, this case is important as it reports the possible effect of GCT on the development of MTs and suggests not to ignore this disease in a dog with mammary tumours.

#### REFERENCES

- Ball, R.L., Birchard, S.J., May, L.R., Threlfall, W.R., Young, G.S., 2010.** Ovarian remnant syndrome in dogs and cats: 21 cases (2000-2007). *Journal of American Veterinary Medical Association* 236, 548-553.
- de las Mulas, J.M., Millan, Y., Dios, R., 2005.** A Prospective Analysis of Immunohistochemically Determined Estrogen Receptor  $\alpha$  and Progesterone Receptor Expression and Host and Tumor Factors as Predictors of Disease-free Period in Mammary Tumors of the Dog. *Veterinary Pathology* 42, 200-212.
- Diez-Bru, N., Garcia-Real, I., Martinez, E.M., Rollan, E., Mayenco, A., Llorens, P., 1998.** Ultrasonographic appearance of ovarian tumors in 10 dogs. *Veterinary Radiology & Ultrasound* 39, 226-233.
- Feldman, E.C., Nelson, R.W., 1987.** Canine female reproduction. In: Feldman, E.C., Nelson, R.W. (Eds.), *Canine and Feline Endocrinology and Reproduction*. WB Saunders, pp. 399-480.
- Genuth, S.M., 1998.** The reproductive glands. In: Berne, R.M., Levy, M.N. (Eds.), *Physiology*. Mosby, Inc, St. Louis, pp. 965-1013.
- Geraldes, M., Gartner, F., Schmitt, F., 2000.** Immunohistochemical study of hormonal receptors and cell proliferation in normal canine mammary glands and spontaneous mammary tumours. *Veterinary Record* 146, 403-406.
- Johnston, S.D., Root Kustritz, M.V., Olson, P.N.S. 2001.** Disorders of the canine ovary. *Ovarian remnant syndrome*. In: *Canine and Feline Theriogenology*, W.B. Saunders, Philadelphia, pp. 199-200.
- Kennedy, P.C., Cullen, M., Ewards, J.F., Goldschmidt, M.H., Larsen, S., Munson, L., Nielsen, S., 1998.** Histological classification of tumors of the genital system of domestic animals. In: Kennedy P.C. (Ed.), *World Helath Organization, International histological classification of tumors of domestic animals*. American Registry of Pathology, Washington D.C., pp. 24-27.
- Kennedy, P.C., Miller, R.B., 1993.** The female genital system. In: Jubb, K.V.F., Kennedy, P.C., Palmer, N. (Eds.): *Pathology of Domestic Animals*. Academic Press, San Diego, pp. 349-470.
- Klein, M.K., 2001.** Tumours of the female reproductive system: In: Withrow, S.J., MacEwen, E.G., (Eds.), *Small Animal Clinical Oncology*. W.B. Saunders, pp. 445-454.
- Lavalle, G.E., Bertagnolli, A.C., Tavares, W.L., Cassali, G.D., 2009.** Cox-2 expression in canine mammary carcinomas: correlation with angiogenesis and overall survival. *Veterinary Pathology* 46, 1275-1280.
- Pluhar, G.E., Memon, M.A., Wheaton, L.G., 1995.** Granulosa cell tumour in an ovariectomized dog. *Journal of American Veterinary Medical Association* 207, 1063-1065.
- Rivera, P., Melin, M., Biagi, T., Fall, T., Haggstrom, J., Lindblad-Toh, K., von Euler, H., 2009.** Mammary tumor development in dogs is associated with BRCA1 and BRCA2. *Cancer Research* 69, 8770-8774.
- Rutteman, G.R., 1990.** Hormones and mammary tumour disease in the female dog: an update. *In vivo*, 4, 33-40.
- Schaefer-Okkens, A.C., Ettinger, S.N., Feldman, E.C., 2005.** Estrous cycle and breeding management of the healthy bitch. In: Fathman, L. (Eds.), *Textbook of Veterinary Internal Medicine*. Elsevier-Saunders, St-Louis, pp. 1640-1649.
- Sleekx, N., de Rooster, H., Veldhuis Koreze, E.J.B., Van Ginneken, C., Van Brantegen, L., 2011.** Canine Mammary tumours, an overview. *Reproduction in Domestic Animals* 46, 1121-1131.
- Sorenmo, K.U., Shofer, F.S., Goldschmidt, M.H., 2000.** Effect of spaying and timing of spaying on survival of dogs with mammary carcinoma. *Journal of Veterinary Internal Medicine* 14, 266-270.
- Spoor, M.S., Flesner, B.K., Trzil, J.E., Whitney, M.S., Shaw, D.P., Selting, K.A., 2014.** What is your diagnosis? Intra-abdominal mass in a female spayed dog. *Veterinary Clinical Pathology* 43, 109-110.
- Tavasoli, A., Solati, A., 2011.** Granulosa cell tumor of the ovary in dog: case report from Tehran. *Journal of Cell and Animal Biology* 5, 66-68.
- Tunca, R., Serin, G., Epikmen, E.T., Aydoğan, A., Avci, H., 2011.** İki Köpekte Granüloza Hücre Tümörü. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 17, 675-678.
- Zanghi, A., Catone, G., Marino, G., Quartuccio, M., Nicotina, P.A., 2007.** Endometrial polypoid adenomyomatosis in a bitch with ovarian granulosa cell tumour and pyometra. *Journal of Comparative Pathology* 136, 83-86.