

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL DIAGNOSIS OF OVARIAN GRANULOSA CELL TUMOUR IN A DOG

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SUMMARY

Granulosa cell tumour morphology is highly variable and creates diagnostic challenges. The present study investigated the morphological pattern, expression of pan-cytokeratin and vimentin in canine granulosa cell tumour. A ten year old female Great Dane was presented with the history of distended abdomen. Histologically the tumour mass consist of round to oval cells with ovoid nucleus arranged in sheets and radially around the small blood vessels. Immuno-histologically, the tumour cells were positive for pan-cytokeratin and negative for vimentin. Based on histology and immunohistochemistry the tumour was diagnosed as granulosa cell tumour.

Keywords: Dog, Granulosa cell tumour, Pan-cytokeratin, Vimentin

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Canine ovarian neoplasms are categorized into four types depending on cell origin, epithelial tumours, germ cell tumours, sex cord stromal tumours and mesenchymal tumours (Agnew and Mac Lachlan, 2016). Among the ovarian tumours epithelial origin (46%) were more common than sex cord stromal (34%) and germ cell origin (20%) (Patnaik and Greenlee, 1987). Granulosa cell tumours (GCTs) are relatively uncommon in canines compared to equines and bovines. This tumour arises from sex-cord stromal cells and observed in bitches along with other types of sex-cord stromal tumours (Perez-Marin *et al.*, 2014; Kamdi *et al.*, 2023). The GCTs are mostly round to spherical in shape, smooth surfaced and the size ranging from 20 to 30 cm diameter (Foster, 2006).

GCTs secrete more progesterone, estradiol and alpha-inhibin (Pluhar *et al.*, 1995), which results in swelling of vulva with vaginal discharge, persistent estrus, alopecia (Buijtsels *et al.*, 2010) and vaginal prolapse (Nak *et al.*, 2012). Histologically variety of growth patterns were observed in GCTs which includes macrofollicular, microfollicular, diffuse, trabecular and Sertoli-like, which is reported both in dogs and human beings. (Scully, 1999; Riccardi *et al.*, 2007). Granulosa cell tumours are very heterogeneous in both humans and dogs, which leads to diagnostic challenges. While immunohistochemistry (IHC) is widely used in human pathology to diagnose granulosa cell tumour, limited studies are available in dogs (Riccardi *et al.*, 2007). The present study record the histological and immunohistochemical characters of granulosa cell tumour in a dog.

A ten year-old female Great Dane was presented to

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treatment with the complaint of recurrent mammary tumour and distended abdomen. The hemato-biochemical values were within normal range except slight monocytosis. The intra abdominal mass was surgically removed and fixed in 10 percent formalin for histopathological examination. The formalin fixed tissue sample was processed, embedded in paraffin wax then sections were taken at 4 µm thickness and stained in Haematoxylin and Eosin (H&E). Another set of tissue sections were taken in poly L lysine coated slides and subjected to pan-cytokeratin and vimentin staining. The IHC was performed as per the standard protocol described by manufacturer (Pathn Situ).

As per the published literature the epithelial ovarian tumours are more common than germ cell tumours and sex cord stromal tumours (Patnaik and Greenlee, 1987). The mean age for ovarian neoplasia in dogs were 10 years and ranging from 10 to 14 years, except with teratoma. The mean age of teratoma was 4 years and ranging from 2 to 9 years (Patnaik and Greenlee, 1987). In the current study, the age of the dog was 10 years, this is well agreed with the findings of Patnaik and Greenlee (1987). Clinical examination revealed distended abdomen and serosanguineous discharge from the edematous vulva. Generally the dogs with granulosa cell tumour showed distended abdomen, lethargy, depression, vulvar swelling with bleeding, enlarged nipples, pyometra, cystic endometrial hyperplasia, persistent estrus and ascites (Zanghi *et al.*, 2007; Goto *et al.*, 2021). In Indian scenerio, invariably ovarian granulosa cell tumours are associated with pyometra (Khokhar *et al.*, 2022; Kumar *et al.*, 2024; Narwade *et al.*, 2024) this might be due to lack of awareness regarding ovariohysterectomy



Fig. 1. Granulosa cell tumour- Firm grey mass with cystic spaces.

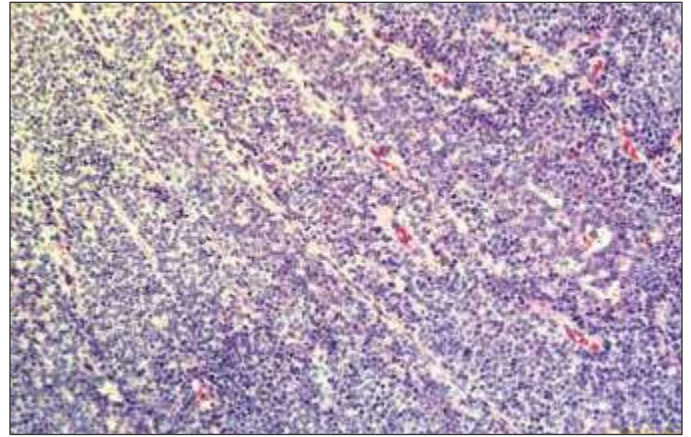


Fig. 2. Granulosa cell tumour showing sheet of neoplastic cells with minimal stroma, H&E x 100.

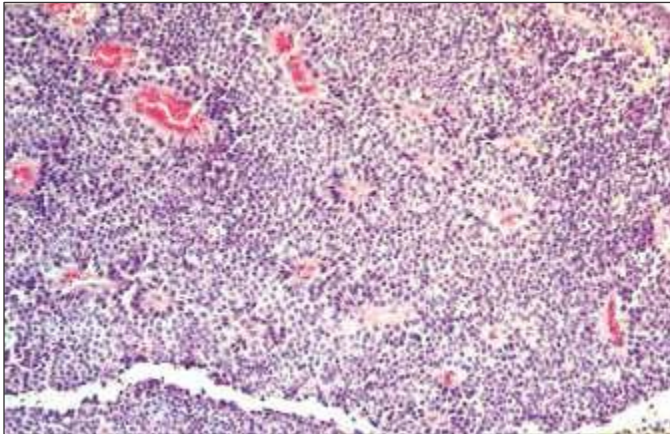


Fig. 3. Neoplastic cells are arranged radially around the blood vessels, H&E x 100.

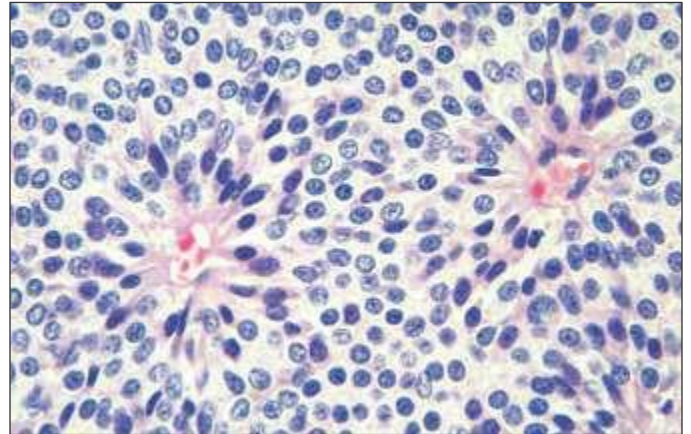


Fig. 4. Higher magnification of Fig. 3 H&E x 400.

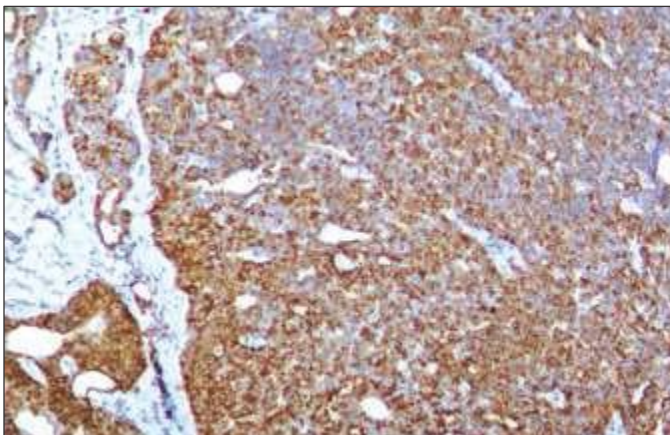


Fig. 5. Neoplastic cells showing positive reaction to pan-cytokeratin IHC x 100.

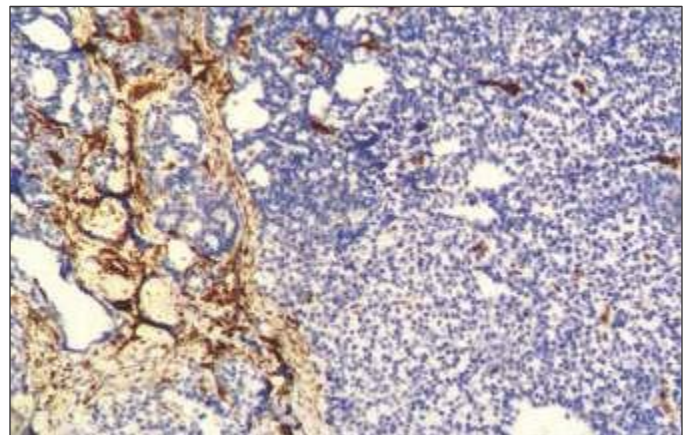


Fig. 6. Neoplastic cells are negative for vimentin but stromal cells are positive. IHC x 100.

practice followed in dogs. Further the retrospective studies on the clinical diagnostic aspects and biomarker analysis in ovarian granulosa cell tumours are limited in India.

On physical examination, firm multiple palpable masses were appreciable in the mid abdomen and a firm irregular mass was seen in the left caudal mammary gland. The gross examination revealed a firm mass about 15×25 cm in size with cystic space. The cut surface was gray to

brown in colour (Fig. 1). The ovarian neoplasms were mostly unilateral (84 percent) except for Sertoli-Leydig cell tumours, which were bilateral nature (36 percent) (Patnaik and Greenlee, 1987) where as Chung *et al.* (2013) reported a rare bilateral ovary granulosa cell tumour with complex mammary carcinoma in a poodle dog. Histologically the tumour mass consist of sheet of cells with minimal stroma (Fig. 2) and follicular patterns. The

tumour cells are round to oval, centrally placed, ovoid nucleus with moderate amount of eosinophilic cytoplasm. In few areas, the neoplastic cells are arranged radially around the small blood vessels called as palisade type (Fig. 3 & 4). The central portion of tumour mass had haemorrhages and necrosis. The histological features were consistent with the findings of Sivacolundhu (2001), Oviedo-Peñata *et al.* (2020) and Kamdi *et al.* (2023).

The neoplastic granulosa cells showed moderate positive reaction for pan-cytokeratin and negative for vimentin. This correlated with the finding of Riccardi *et al.* (2015) who reported that cytokeratin AE1/AE3 was positive for all epithelial derived ovarian tumours and 6 of 8 granulosa cell tumours where as granulosa and epithelial cell tumours showed variable expression pattern for vimentin. Another study suggested that most of the sexcord stromal tumours were positive for AE1/AE3 except for palisade type (Akihara *et al.*, 2007). Granulosa cells and associated tumours were invariably negative for HBME-1, however 17 out of 18 ovarian epithelial cancers and normal ovarian surface epithelium tested positive (Banco *et al.*, 2011). There are controversies in selection of IHC marker panel for differentiating the ovarian tumours in dogs so a comprehensive study is required.

Based on the histological feature of palisade arrangement of neoplastic cells around the blood vessels and positive reaction of neoplastic cells to pan-cytokeratin by immuno-histochemistry the tumour was confirmed as granulosa cell tumour.

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