Case Report

jfms OPEN REPORTS

Journal of Feline Medicine and Surgery Open Reports
1–5

DOI: 10.1177/2055116917722701
journals.sagepub.com/home/jfmsopenreports

This paper was handled and processed by the European Editorial Office (ISFM) for publication in *JFMS Open Reports*

SAGE

Serum anti-Müllerian hormone concentrations before and after treatment of an ovarian granulosa cell tumour in a cat

Laura A Heaps[1], Christopher J Scudder[1,2], Vicky J Lipscomb[1], Sarah ML Steinbach[3], Simon L Priestnall[4], Henny Martineau[4], Balazs Szladovits[4], Robert C Fowkes[2] and Oliver A Garden[1]

Abstract

Case summary A 15-year-old female cat was presented for investigation of progressive behavioural changes, polyuria, polydipsia and periuria. An ovarian granulosa cell tumour was identified and the cat underwent therapeutic ovariohysterectomy (OHE). The cat's clinical signs resolved, but 6 months later it was diagnosed as having an anaplastic astrocytoma and was euthanased. Serum anti-Müllerian hormone (AMH) concentration prior to OHE was increased vs a control group of entire and neutered female cats. Following OHE, serum AMH concentration decreased to <1% of the original value.

Relevance and novel information Serum AMH measurement may represent a novel diagnostic and monitoring tool for functional ovarian neoplasms in cats.

Accepted: 26 June 2016

Case description

A 15-year-old female domestic shorthair cat was presented for investigation of an 8 month history of progressive behavioural changes (episodic pacing and vocalisation), polyuria, polydipsia and periuria.

At presentation the cat had a body weight of 1.98 kg and a body condition score (BCS) of 3/9. Physical examination revealed diffuse seborrhoea sicca and a palpable (approximately 10 mm diameter) mid-dorsal abdominal mass. Neurological and fundic examinations were within normal limits. Mean systolic blood pressure measured by Doppler sphygmomanometry was 176 mmHg.

Clinicopathological abnormalities included mild lymphopenia (0.6×10^9/l; reference interval [RI] 1.5–7.0 $\times 10^9$/l) and marginal microcytosis (38.3 fl; RI 39.0–55.0 fl). Serum biochemical analysis was unremarkable. Serum feline immunodeficiency virus and feline leukaemia virus immunochromatographic tests were both negative. Urinalysis of a cystocentesis sample yielded a specific gravity of 1.028 and urine dipstick test results were unremarkable. Urine protein:creatinine ratio was within the RI (0.25; RI 0–0.50), urine sediment examination was unremarkable and urine bacterial culture was negative. Prothrombin time was mildly increased (12.7 s; RI 7.0–11.0 s); activated partial thromboplastin and buccal mucosal bleeding times were within the RIs.

[1]Department of Clinical Science and Services, Royal Veterinary College, North Mymms, UK
[2]Endocrine Signalling Group, Comparative Biomedical Sciences, Royal Veterinary College, London, UK
[3]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN, USA
[4]Pathobiology and Population Sciences, Royal Veterinary College, North Mymms, UK

Corresponding author:
Christopher J Scudder BVSc, MVetMed, MRCVS, DACVIM-SAIM, Queen Mother Hospital for Animals, Royal Veterinary College, Hawkshead Lane, North Mymms, Hertfordshire, AL9 7TA, UK
Email: cscudder@rvc.ac.uk

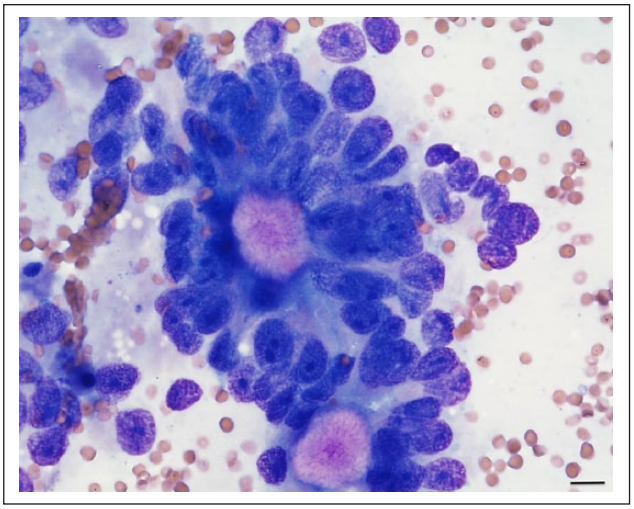

Figure 1 Fine-needle aspirate cytology from the right ovary. Epithelial cells arranged in acinar-like structures with bright eosinophilic granular material in the middle (Call–Exner bodies); × 40 oil objective, Modified Wright's stain. Bar = 10 µm

Abdominal ultrasound examination revealed heterogenous enlargement of the right ovary (11 mm diameter), the uterine wall thickness was normal and the uterine lumen contained a small volume of anechoic material. Cytological review of fine-needle aspirates from the right ovary found epithelial cells commonly arranged in acinar-like structures with bright eosinophilic granular material in the middle, consistent with Call–Exner bodies (Figure 1). These findings were suggestive of a granulosa cell tumour (GCT).[1] An aspirate of the anechoic uterine material revealed a total nucleated cell count of 0.2 × 10^9/l, fluid total protein of 1.8 g/l and cytological review of the fluid identified 69% poorly preserved neutrophils, 3% lymphocytes, 27% macrophages and 1% eosinophils consistent with slight neutrophilic and macrophagic inflammation. Bacterial culture of the uterine fluid was negative. Contrast-enhanced CT (Mx8000 IDT; Philips) of the brain, abdomen and thorax did not reveal evidence of gross metastatic disease. A 4 mm hypoattenuating irregularly marginated lesion was present within the parenchyma of the right liver lobe; ultrasound-guided fine-needle aspiration of the lesion yielded only occasional clusters of hepatocytes with no evidence of metastatic disease.

The cat subsequently underwent therapeutic ovariohysterectomy (OHE), surgical biopsy of the liver lesion and biopsy of a regional lymph node to the uterus. Histopathological examination of the right ovary revealed a partially encapsulated, multi-lobular, densely cellular proliferation of neoplastic cells that almost completely effaced the normal architecture (Figure 2a). There was moderate anisocytosis and anisokaryosis, and two mitotic figures observed in 10 high power (× 400) fields. Occasional malformed Call–Exner bodies were seen and there were focal areas composed of theca cells. In one area towards the periphery of the ovary there was a large cluster of neoplastic cells within a thick-walled vessel, which raised concern for vascular invasion. The sections of examined uterus exhibited glandular cysts of varying sizes within the endometrium but no evidence of inflammation. Histopathological examination of the liver biopsy revealed diffuse, mild, periportal infiltrate of lymphocytes and plasma cells accompanied by a mild proliferation of cells with oval nuclei and a small amount of fibrous connective tissue. The periacinar hepatocytes were often mildly atrophic with thinning of cords and relative widening of sinusoids. There was mild hyperplasia of Ito cells and occasional lipogranulomas were also present. Multifocally, hepatocytes contained stippled brown intracytoplasmic material (lipofuscin), and rare plugging of bile canaliculi was also present. These changes were consistent with mild periacinar hepatocellular atrophy and mild chronic lymphoplasmacytic portal hepatitis. Examination of the lymph node biopsy revealed mild sinus histiocytosis and the tissue was otherwise unremarkable.

Immunohistochemistry staining for anti-Müllerian hormone (AMH) was performed as previously described.[2] A citrate buffer with microwave heating antigen retrieval was performed; the primary antibody was polyclonal rabbit anti-human AMH (Abcam ab84415) and immunoreactivity was visualised using biotinylated goat anti-rabbit antibody (Vectastain Elite ABC HRP Rabbit IgG; Vector Laboratories) and DAB (DAB Peroxidase [HRP] Substrate Kit; Vector Laboratories). AMH immunoreactivity was identified in developing follicles as described in humans, and within the neoplastic population of cells in a similar pattern as described for equine GCTs (Figures 2b–e).[2,3] Findings of ovarian histopathology, including the degree of cellular pleomorphism, high nuclear to cytoplasmic ratio and size of the neoplasm, in addition to the immunohistopathological findings of vascular invasion, resulted in a diagnosis of malignant GCT.

Prior to surgery, hormone testing was undertaken to evaluate whether the tumour was endocrinologically active. A dexamethasone suppression test (0.1 mg/kg IV dexamethasone) revealed suppression of serum cortisol after 3 and 8 h (basal serum cortisol concentration 201.0 nmol/l; 3 and 8 h serum cortisol concentrations were both <27.6 nmol/l). Plasma endogenous adrenocorticotropic hormone assay (>600 pg/ml; RI 38–176 pg/ml). These results provided no evidence for excessive glucocorticoid production by the tumour.

Serum oestradiol, progesterone and testosterone concentrations were within normal limits (Table 1). Serum AMH concentration was measured by a commercial laboratory (NationWide Specialist Laboratories) using sandwich ELISA for the measurement of AMH in horses. Serum AMH was 5.7 ng/ml prior to OHE, whereas the

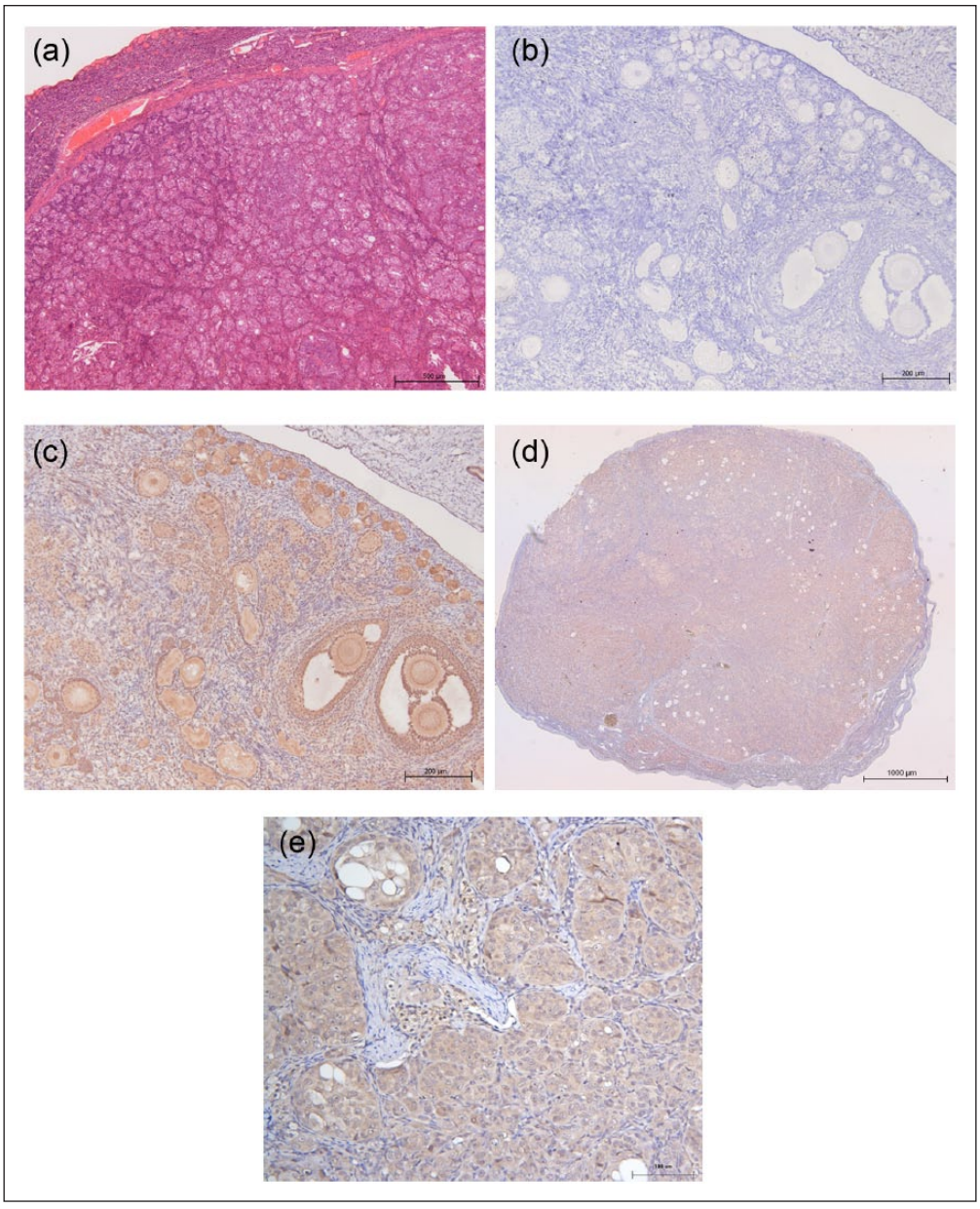

Figure 2 (a) Haematoxylin and eosin-stained section of tissue of the ovarian neoplasm showing a region where the neoplasm is encapsulated. (b–e) Anti-Müllerian hormone immunoreactivity. (b) Antibody-negative control section of tissue from a healthy ovary. (c) Anti-Müllerian hormone immunoreactivity identified in primordial, primary and secondary ovarian follicles from a healthy ovary. (d) × 50 magnification, (e) × 200 magnification image demonstrating anti-Müllerian hormone immunoreactivity identified within the neoplastic population of granulosa cells

Table 1 Results of serum sex hormone analysis prior to ovariohysterectomy

	Patient serum concentration	Upper limit of reference interval
Oestradiol (pmol/l)	5.1	10.0
Progesterone (pmol/l)	2.5	3.0
Testosterone (nmol/l)	0.15	0.2

highest concentration measured in a group of healthy neutered (n = 8) and entire female (n = 4) cats was 1.7 ng/ml (Figure 3). Two months following OHE, serum AMH concentration had decreased to <0.04 ng/ml.

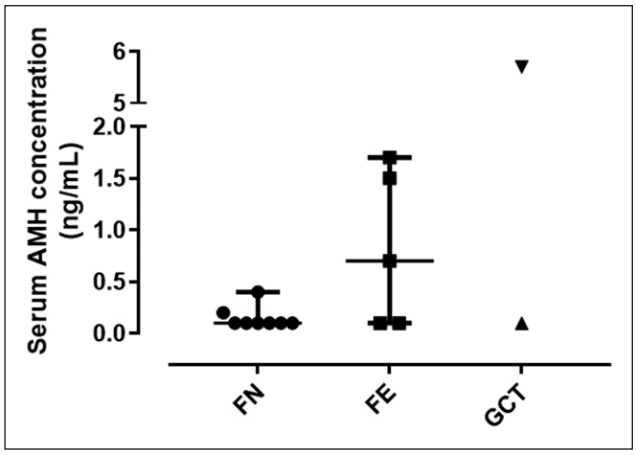

Figure 3 Scatter dot plot representing serum anti-Müllerian hormone (AMH) concentration (ng/ml) in female neutered (FN; n = 8) and female entire (FE; n = 4) control groups, and pre-(inverted triangle) and post-ovariohysterectomy (triangle) in the cat diagnosed with a granulosa cell tumour (GCT). The length of the vertical line represents the range of the data and the dissecting line represents the median of the data. The lower range and median lines are superimposed in the FN group. The lower level of detection of the assay is 0.04 ng/ml

Surgical intervention resulted in complete resolution of clinical signs and BCS increased to 4/9 at re-evaluation 1 month later. A complete blood cell count and serum biochemistry performed at this time were unremarkable. However, 6 months following surgery the cat presented to the primary veterinary surgeon following several complex partial seizures. These episodes manifested as facial twitching, vocalisation, falling and circling to the right. The cat later developed central blindness and was euthanased.

Post-mortem macroscopic examination did not reveal any gross abnormalities. Microscopic examination of the brain revealed a neoplasm expanding and effacing the parenchyma within the neocortex above the midthalamus. The neoplasm was well-demarcated, nonencapsulated, densely cellular and was composed of numerous astrocytes exhibiting moderate anisocytosis, anisokaryosis and mitotic activity. Large areas of necrosis were present and neoplastic astrocytes widely infiltrated the adjacent neuropil. The neoplasm exhibited positive immunoreactivity for vimentin and glial fibrillary acidic protein consistent with an intermediate grade (anaplastic) astrocytoma.

Discussion

Ovarian neoplasms are rare in UK cats. This is likely to reflect the predominantly neutered population of female cats in the UK. GCTs are stromal tumours arising from ovarian granulosa cells and represent nearly half of all ovarian neoplasms in cats.[4] These tumours predominantly occur in middle-aged-to-geriatric cats.[5] Metastatic behaviour shows considerable interspecies variation, occurring rarely in mares but commonly in queens, with reported metastasis in around 50% of cases.[4] Metastasis occurs to local lymph nodes, peritoneum or haematogenously to distant organs.[6]

Some GCTs produce functional hormones, including glucocorticoids and reproductive hormones such as oestrogen, progesterone, testosterone and inhibin.[6] Aberrant reproductive hormone production can result in persistent anestrus, continuous oestrus or masculinisation.[4,6] The diagnosis of both a GCT and an astrocytoma in the same patient raises the question of whether the astrocytoma was contributing to the initial presenting clinical signs. We believe the cat's initial behavioural changes were a consequence of GCT-induced oestrous behaviour because the cat's clinical signs resolved following OHE and because CT of the brain was unremarkable at initial presentation. However, it is plausible that it had abdominal pain, which was not appreciated during initial physical examination and which might have contributed to its initial behaviour aberrations.

The hormone AMH is a dimeric glycoprotein in the transforming growth factor β superfamily, which has not been previously measured in cats with ovarian neoplasms. AMH is required for male gonadogenesis and is secreted by granulosa cells in pre-antral and small follicles at low concentrations in adult female horses.[3] In this role, AMH inhibits the recruitment of primordial follicles into the growing pool by reducing the responsiveness of follicles to follicle-stimulating hormone and thus limiting the number of actively growing follicles.[7] A recent study described serum AMH as a diagnostic test with 100% sensitivity and specificity for the differentiation between neutered and entire status in cats.[8] The presence of AMH in primordial, primary and secondary feline follicles was demonstrated for the first time by the immunohistochemical analyses. The presence of AMH in early feline follicles suggests the inhibitory role of follicular development by AMH might also occur in cats.

Serum inhibin has previously been used as the bioassay of choice to detect functional GCTs in women and mares.[9–12] However, serum AMH is now considered to be a more specific marker for GCTs than inhibin, with documented increases in serum AMH concentrations in 76–93% of women and in 100% of mares with GCTs.[13–17] The results of serum AMH prior to surgery in this case support the use of this test in the diagnosis of feline GCTs. Serum AMH might also be useful for the monitoring of recurrence of feline GCTs because these tumours can recur years after the original diagnosis in humans.[18,19]

The concurrent ovarian and uterine abnormalities seen in this case were not unexpected. Endometrial hyperplasia has been previously reported in a cat and in dogs with a GCT.[20,21] Cystic endometrial hyperplasia is most commonly a consequence of oestrogenic effects on the uterus. Increased serum progesterone may also induce endometrial changes; however, the histopathological appearance

is different. Oestrogenic stimulation results in a cuboidal-to-low-columnar endometrial epithelium, whereas progesterone induces tall columnar epithelium with extensive vacuolation.[6] Serum oestradiol measurement in women is an unreliable marker for GCTs because it is only increased in 70% of cases.[18] In the current case, while serum progesterone and oestradiol concentrations were not increased, the uterus could still have been under the influence of local oestrogens.

Conclusions

We suggest that serum AMH may be implicated in the pathophysiology of functional GCTs in cats. Serum AMH could also represent a novel hormone test to assist the diagnosis of functional feline ovarian neoplasms and to assess response to treatment and monitoring for tumour recurrence.

Authors' note We report no off-label antimicrobial use.

Acknowledgements The authors would like to acknowledge Clair Muir and Helen Evans for their contributions to this case report.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding The authors received no financial support for the research, authorship, and/or publication of this article.

References

1 Norris HJ, Garner FM and Taylor HB. **Pathology of feline ovarian neoplasms**. *J Pathol* 1969; 97: 138–143.
2 Weenen C, Laven JSE, von Bergh ARM, et al. **Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment**. *Mol Hum Reprod* 2004; 10: 77–83.
3 Ball BA, Conley AJ, MacLaughlin DT, et al. **Expression of anti-Müllerian hormone (AMH) in equine granulosa-cell tumors and in normal equine ovaries**. *Theriogenology* 2008; 70: 968–977.
4 Gelberg HB and McEntee K. **Feline ovarian neoplasms**. *Vet Pathol* 1985; 22: 572–576.
5 Saba CF and Lawrence JA. **Tumors of the female reproductive system**. In: Withrow SJ and Vail DM (eds). Withrow and MacEwen's small animal clinical oncology. 5th ed. St Louis, MO: Elsevier Saunders, 2013, pp 532–537.
6 MacLachlan NJ and Kennedy PC. **Tumors of the genital systems**. In: Meuten DJ (ed). Tumors in domestic animals. 4th ed. Ames, IA: Iowa State Press, 2008, pp 547–573.
7 Durlinger AL, Visser JA and Themmen AP. **Regulation of ovarian function: the role of anti-Mullerian hormone**. *Reproduction* 2002; 124: 601–609.
8 Axnér E and Ström Holst B. **Concentrations of anti-Müllerian hormone in the domestic cat. Relation with spay or neuter status and serum estradiol**. *Theriogenology* 2015; 83: 817–821.
9 Lappöhn RE, Burger HG, Bouma J, et al. **Inhibin as a marker for granulosa-cell tumors**. *N Engl J Med* 1989; 321: 790–793.
10 Hildebrandt RH, Rouse RV and Longacre TA. **Value of inhibin in the identification of granulosa cell tumors of the ovary**. *Hum Pathol* 1997; 28: 1387–1395.
11 Bailey MT, Troedsson MHT and Wheaton JE. **Inhibin concentrations in mares with granulosa cell tumors**. *Theriogenology* 2002; 57: 1885–1895.
12 Ball BA, Conley AJ, Almeida J, et al. **A retrospective analysis of 2,253 cases submitted for endocrine diagnosis of possible granulosa cell tumors in mares**. *J Equine Vet Sci* 2014; 34: 307–313.
13 Rey RA, Lahlou N, Bidart JM, et al. **Antimüllerian hormone as a serum marker of granulosa cell tumors of the ovary: comparative study with serum α-inhibin and estradiol**. *Am J Obstet Gynecol* 1996; 174: 958–965.
14 Rey R, Sabourin JC, Venara M, et al. **Anti-mullerian hormone is a specific marker of sertoli- and granulosa-cell origin in gonadal tumors**. *Hum Pathol* 2000; 31: 1202–1208.
15 Geerts I, Vergote I, Neven P, et al. **The role of inhibins B and antimüllerian hormone for diagnosis and follow-up of granulosa cell tumors**. *Int J Gynecol Cancer* 2009; 19: 847–855.
16 La Marca A and Volpe A. **The anti-Mullerian hormone and ovarian cancer**. *Hum Reprod Update* 2007; 13: 265–273.
17 Almeida J, Ball BA, Conley AJ, et al. **Biological and clinical significance of anti-Müllerian hormone determination in blood serum of the mare**. *Theriogenology* 2011; 76: 1393–1403.
18 Pectasides D, Pectasides E and Psyrri A. **Granulosa cell tumor of the ovary**. *Cancer Treat Rev* 2008; 34: 1–12.
19 Long WQ, Ranchin V, Pautier P, et al. **Detection of minimal levels of serum anti-Mullerian hormone during follow-up of patients with ovarian granulosa cell tumor by means of a highly sensitive enzyme-linked immunosorbent assay**. *J Clin Endocrinol Metab* 2000; 85: 540–544.
20 Patnaik AK and Greenlee PG. **Canine ovarian neoplasms: a clinicopathologic study of 71 cases, including histology of 12 granulosa cell tumors**. *Vet Pathol* 1987; 24: 509–514.
21 Giacoia MR, Maiorka PC, Oliveira CM, et al. **Granulosa cell tumour with metastasis in a cat**. *Brazilian J Vet Res Anim Sci* 1999; 36: 250–252.