## **Successful intra-abdominal resection of a 24 kg ovarian granulosa cell tumor in a Warmblood mare**

## **ABSTRACT**

## Granulosa cell tumor (GCT) is a benign tumor that affects a mare’s ovaries. In this report, a case of unilateral GCT in an ovary, which weighed 24 kg, of a 7-year-old warmblood mare is described, including ultrasonographic, clinical appearance and successful surgical removal of the tumor. The GCT was the cause of an acute hemoperitoneum and after a first stabilization of the mare the GCT was removed through a ventral midline incision. Because of the enormous dimensions of the GCT, intra-abdominal partial resection of the tumor using a tenotomy knife was performed to exteriorize the ovarian pedicle. The technique can cause an intra-abdominal hemorrhage and the time between GCT resection and sealing of the respective ovarian pedicle should be as short as possible. After 3.5 months, the mare had a good cosmetic and functional outcome and was ridden for her intended use.

## Metastasis of GCT is rare, but not impossible. The mare therefore should be followed up for a longer period of time to investigate if metastasis develop.

## **Introduction**

Granulosa cell tumours (GCTs) are the most common ovarian tumours in mares (Meagher et al. 1977; Sundeber et al. 1990; McCue et al. 2006), representing >85% of reproductive neoplasms (McCue et al. 1998) and approximately 2.5% of all neoplasms in horses (Sundeber et al. 1990). All GCTs derive from the follicular granulosa cells (Kennedy et al. 1998), with a subset of granulosa-theca cell tumours (GTCTs) also comprising a distinct theca-derived component (Kennedy et al. 1998) that can be demonstrated structurally by histopathology and functionally by a capacity for androgen secretion (Neto et al. 2010). The classical presentation of a GCT is a unilaterally enlarged ovary lacking a palpable ovulation fossa, and a small, inactive contralateral ovary. In addition, GCTs can appear as a multicystic (‘honeycomb’) mass (>83% of cases) (Murase et al. 2018; Sherlock et al. 2018), or less often as a solid ovarian mass (7% of cases) (Sherlock et al. 1990) or even a single fluid-filled cyst (Hinrichs et al. 1190; McCue et al. 1998; Renaudin et al. 2021). Affected mares may exhibit prolonged anoestrus, continuous or intermittent oestrus (nymphomania behaviour) or aggressive, stallion-like behaviour (Meagher et al. 1977; Neto et al. 2010; Hinrichs et al. 1990a). There is no definitive diagnostic test for GTCTs and GCT in horses, and identification is usually based on the history, rectal examination, ultrasonographic examination and serum hormone analysis (Christman et al. 1999; Hinrichs et al. 1990b). Surgical removal of the affected ovary is the treatment of choice, Meagher et al. 1997; Perino et al. 1985) as these tumours rarely metastasize (Meagher et al. 1997; Perino et al. 1985; Bosu et al. 1982). Rarely, GCTs and GTCTs have been reported to cause hemoperitoneum as a result of rupture prior to or during surgery (Alexander et al. 2004; Harper et al. 2010; Sherlock et al. 2016; Worsman et al. 2018). This rupture may be caused by rapid growth of the tumour. Patient presentation of a haemorrhagic GCT is nonspecific and does not always present as haemorrhagic shock (Hinrichs et al. 1990a; Renaudin et al. 2021; Crabtree et al. 2011). Due to the lack of specificity in clinical symptoms, it is difficult to arrive at a definitive diagnosis before surgery, but GCTs should be considered as a differential diagnosis in case of hemoperitonaeum in mares. The treatment of choice for removal of a GCT is a laparascopic ovariectomy in the standing mare. Ovariectomy under general anaesthesia is preferred in horses with big GCTs that cannot be removed via a flank laparotomy.

This case report describes a warmblood mare with acute intra-abdominal haemorrhage caused by a 25kg GCT that was removed by a ventral midline laparotomy.

## **Initial clinical presentation and treatment**

A 7-year-old Warmblood mare was referred to the Department for Horses because of a suspected impaction of the ascending colon. On initial examination, the mare showed tachycardia (60 beats/min), tachypnea (40 breaths/min) and fever (38.7°C). She had pale mucous membranes and a prolonged capillary refill time of more than three seconds. Borborygmi were decreased and she showed mild to moderate abdominal discomfort. Transabdominal ultrasonography revealed a large amount of echogenic, swirling fluid consistent with blood (Fig. 1). Haemoabdomen was confirmed by abdominocentesis. Transrectal palpation revealed a firm structure in the ventral abdominal cavity, which extended across the abdomen from the right to the left side. This structure was initially suspected to be an impaction. Nasogastric intubation yielded no reflux.

Hematologic examination showed profound anaemia (PCV 14%, reference interval 27 – 39%, RBC 2.79 T/l, reference interval 6.6-9.8 T/l, haemoglobin 2.9 mmol/l, reference interval 7.2-10.2 mmol/l) and leucocytosis (15 G/l, reference interval 4.4-12 G/l), which was characterised by neutrophilia (13,95 G/l, reference interval 1,6-8.7 G/l). The mare also showed a hypoproteinaemia (54.7 g/l, reference interval 57.8-78.7g/l) and a suspected pre-renal azotaemia (Creatinine: 249 µmol/l, reference interval 83.7-156.4 µmol/l, blood urea nitrogen (BUN) 22.77 mmol/l, reference interval 2.51-7.34 mmol/l). Concentrations of cholesterol (3.5 mmol/l, reference interval 1.72-2.95mmol/l) and triglycerides (4.82mmol/l, reference interval 0.13-0.61 mmol/l) as well as the activities of creatine kinase (4873 U/l, reference interval 146-354), lactate dehydrogenase (2182 U/l, reference interval 223.9-536.3 U/l), and aspartate amino transferase (1597, reference interval 213.2-626.7) were elevated. Minor electrolyte derangements were present.

The mare was stabilised with crystalloid fluid therapy (Ringer’s Lactate) and received a blood transfusion. After transfusion of approximately 450 ml of blood, the mare developed a cardiac arrhythmia that resolved without additional treatment after pausing the transfusion. The arrhythmia had resolved by the time the ECG was placed. The remainder of the transfusion (1.2L) was administered without further complications under ECG monitoring. The mare was cautiously treated with laxatives for the suspicious of colon impaction. Because of the risk of the sepsis, the mare received metaphylactic antibiotics (amoxicillin 10 mg/kg BID, Amoxicillin-Natrium®, CP-Pharma; gentamicin 6.6 mg/kg SID, Genta®, CP-Pharma), as well as flunixin meglumine (0.55 mg/kg BID, Flunidol®, CP-Pharma), and etamsylat (7 mg/kg TID i.v., hemosilate®, Ecuphar GmbH, Greifswald/Germany).

Over the next days, the haemoabdomen regressed and the mare did not show abdominal discomfort anymore. Sonographically, a mass with a honeycomb structure was visible inguinally on both sides (Fig.1). The firm structure that was palpable transrectally was unchanged, and transrectal ultrasonography suggested that it was the same mass as seen inguinally. Differential diagnoses included a GCT, GTCT’s and hemangiosarcoma. Since the owner declined laparoscopy and biopsy, the blood concentration of anti-muellerian hormone (AMH) was determined and was highly suggestive of a GCT (22.96 ng/ml; reference interval for healthy mares <4 ng/ml, equivocal 4-7 ng/ml, suggestive of granulosa cell tumor >7 ng/ml). The mare was discharged from the hospital 14 days after the initial presentation with the advice of a re-examination and ovariectomy in 4 weeks. At this time, anemia had improved (PCV29%, reference interval 27 – 39%, RBC 5.91 T/l, reference interval 6.6-9.8 T/l, haemoglobin 6.3 mmol/l, reference interval 7.2-10.2 mmol/l) and the amount of intra-abdominal fluid had significantly decreased.

## **Surgical treatment**

Four weeks after hospital discharge, the mare was re-admitted for unilateral ovariectomy. She appeared bright, alert and responsive, and vital parameters were within normal limits. On sonographic examination, a minor amount of free abdominal fluid was evident. The honeycomb-structured mass was visible on both sides of the abdomen, extending from the kidneys to the ventral midline. Haematologic examination showed moderate anaemia (PCV 20%, reference interval 27 – 39%, RBC 4.98 T/l, reference interval 6.6-9.8 T/l, haemoglobin 4.9 mmol/l, reference interval 7.2-10.2 mmol/l).

Preoperatively, the mare received amoxicillin 10 mg/kg BID (Amoxicillin-Natrium®, CP-Pharma), gentamicin (6.6 mg/kg bwt SID i.v., Genta®, CP-Pharma). Systemic analgesia was provided with levomethadonhydrochlorid (0.1 mg/kg, L-Polamivet®, MSD Animal Health) and flunixin meglumine (1.1 mg/kg bwt SID i.v., Flunidol®, CP-Pharma). The mare was premedicated with romifidine (0.08 mg/kg i.v.) and was induced with rapid injection of Diazepam (0.06 mg/kg) in combination with ketamine (2.2 mg/kg i.v.). After induction of anaesthesia, the horse was intubated and positioned in dorsal recumbency. Anaesthesia was maintained with an isoflurane (MAC of ~1.3% c.f.󠅏) and oxygen (MAC of 5 l/min) mixture combined with a constant rate infusion (CRI) of ketamine (2.2 mg/kg/h).

Immediately after a ventral midline incision, the GCT was visible in the surgical field (Fig. 3). It extended across the abdomen from the right to the left side. On the right side, it was adjacent to the caecum and the ascending colon, and small intestine was visible underneath the GCT. Cranially, the GCT extended to the stomach and the caudal part of the lateral right lobe of the liver. Following the caudal surface of the GCT, the association of the GCT to the right ovary was ascertained. The right uterine horn and oviduct were palpable.

Exteriorization of any part of the intestine was not possible due to the dimension and weight of the GCT. The only possibility to exteriorize the GCT was to dissect it. For the procedure, a tenotomy knife was used to transect the GCT in its length and ca. 1/3 of the mass was removed. The dissection of the GCT was performed blindly under hand control. During the dissection the GCT was cut starting from the middle of the GCT, were the risk of cutting organs or bowel was minor. Cutting through the GCT the intestine was protected under the hand to resect part of the GCT in hits length avoid damaging of the bowel. At this time, a bleeding was caused by the partial resection of the GCT. After partial ovariectomy, the GCT and the right ovarian pedicle and right uterine horn were exteriorized (Fig. 4). Using a vessel-sealing device (Ligasure®, Covidien) the blood vessels were sealed and the GCT was removed *in toto* and the ovarian bleeding stopped as soon as the ovarian pedicle was sealed*.* After GCT removal, the abdomen was flushed with 20L sterile saline solution to remove fibrin clots remaining from the previous haemoperitoneum, and to prevent adhesion formation. The midline was closed in four layers and a belly bandage was applied. The mare recovered uneventfully. The removed GCT was found to weigh 24.6 kg and to have a diameter of 107cm. Histopathology confirmed a GCT (Fig. 5).

Due to intraoperative bleeding, the mare developed severe anaemia (PCV13%) and an increased heart rate (68 beats per minute). A blood transfusion was performed (4L). During the transfusion, the mare developed an allergic reaction showing fever and urticaria. The transfusion was stopped and the mare received prednisolone-21-hydrogensuccinate as sodium salt (2 mg/kg, Prednisolut® 250 mg, mibe GmbH Arzneimittel, Brehna/Germany). Vital parameters normalized during the following hour and the transfusion was not continued. During hospitalisation, the mare received antibiotics (amoxicillin 10 mg/kg BID, Amoxicillin-Natrium®, CP-Pharma; gentamicin 6.6 mg/kg SID, Genta®, CP-Pharma), as well as flunixin meglumine (0.55 mg/kg BID, Flunidol®, CP-Pharma) for five days after surgery. The laparotomy incision was well adapted and showed no signs of surgical site infection. The PCV stabilized and was 24% before discharge from the hospital, eight days following surgery.

The findings of the histological examination of the ovary revealed the presence of an extensive necrotic granulosa cell tumour with severe haemorrhages, multifocal fibrinous-purulent inflammation and focal haematoma formation.

## **Outcome and follow-up**

The mare was discharged with recommendations for a total of 6 weeks (including the week of hospitalisation) of stall confinement with only short hand walks (5min daily, increase by 5 min each week). Follow-up information was obtained via E-Mail 6 months after the surgery. The mare was being ridden successfully. The contralateral ovary showed a normal hormonal activity and size. During the follow up period the mare did not show any abdominal discomfort, colic symptoms or stallion behavior.

## **Discussion**

As most common causes of hemoperitoneum in horses neoplasia (approximately 13% of cases), idiopathic hemorrhage (22%), uterine injury (22%), ruptured blood vessels (20%) and splenic lesions (19%) were described (Sherlock et al. 2016). Trauma is thought to explain as many as 9% of cases (Worsman et al. 2018), while hemoperitoneum due to ovarian haematoma or GCT is less common. GCT has been reported to cause hemoperitoneum as a result of rupture prior to or during surgery (Alexander et al. 2004; Harper et al. 2010; Sherlock et al. 2016; Worsman et al. 2018), and it is possible that this rupture occurs due to rapid growth of the tumour. In reported cases of hemoperitoneum caused by GCT, haemorrhage was severe and sometimes life threatening due to systemic cardiovascular compromise.

The treatment of choice for GCT is surgical removal (Alexander et al. 2004; Sherlock et al. 2016; Crabtree et al. 2011). A number of surgical approaches for ovariectomy have been described, and include midline, flank, and paramedian laparotomy, colpotomy and bilateral or unilateral laparoscopic approaches in the standing anaesthetised horse (Smith et al. 2008; Gee et al. 2012; Sinovich et al. 2022; Ragle et al. 2012; Daniel et al. 2015; Colbath et al. 2017). Conservative treatment in some cases was followed by surgical removal at a later stage (Alexander et al. 2004) once medical stabilisation was achieved.

In the case reported, the duration of tumour growth prior to clinical presentation is unknown; however, the clinical appearance would support the theory of Worsman et al. (Worsman et al. 2018) that rupture can occur due to rapid growth of the tumour. The size of the GCT did not permit standing ovariectomy, which is routinely undertaken as a laparoscopic or hand-assisted procedure (Ragle et al. 2012; Daniel et al. 2015). General anaesthesia is always considered a high-risk option in horse, especially in this case in which the horse had an acute hemoperitoneum at the first admission. For this reason, it was important to first stabilize the horse with a blood transfusion and infusions with crystalloid solutions and to perform surgery when the hemoperitoneum partially resolved and the haematocrit had stabilized. Furthermore, with a diagnostic laparotomy, it was possible to exclude adhesions between the GCT and other organs since it permitted better visualization and evaluation of the ovarian pedicle (Harper et al. 2010; Worsman et al. 2018; Conwell et al. 2010; Finding et al. 2011). For safety reasons, the GCT should not be cut, but in this case, this was the best option. One of the main disadvantages of this technique is that the GCT was cut blindly in the abdomen. This is a high-risk technique because the bowel or other organs could be damaged by mistake. To avoid the injury of the intestine or organs, it is important to cut the GCT under hand control, staying in the middle of the tumour, avoiding deep blind cut and trying to feel what the tenotomy knife is cutting. Additionally, once the tumour is partially resected, bleeding is inevitable since the ovarian artery is still in connected with the GCT. In fact, one of the main complications of this technique is the intra operative bleeding after partial ovariectomy. The time between the partial ovariectomy and the sealing of the ovarian pedicle should be minimized to prevent a hypovolemic shock. The surgeon and the anaesthetist should be aware from the complication and needs to have all the instruments ready to stop the bleeding sealing the ovarian pedicle, as well as a blood donor for massive bleeding.

Limitations of this case report are its single-case character and the possibility of metastasis to intra-abdominal GCT splitting. However, based on the previous literature, metastasis of GCT is rare (Meagher et al. 1997b; Perino et al. 1985; Bosu et al. 1982), but long-time follow-up should be pursued, especially to rule out metastasis.

This case report describes an alternative approach for removal of a large GCT with good outcome. The surgical approach included intra-abdominal splitting of the ovarian tumour to permit access to the ovarian pedicle and subsequent removal of the GCT.

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