"An O-Vary Exciting CPC"

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Introduction

  Reproductive disorders in the equine species can be both economically consequential and an important welfare issue for both horse and handler. Granulosa Theca Cell Tumors (GTCT) make up a significant portion of these disorders with a reported prevalence in the United States of >2.5% of all reproductive neoplasms[3,4,9]. This disease is often unilateral, benign, and carries a good prognosis if treated [2,5,6,7,9]. However, with this uniquely benign neoplasm come many not-so benign risks and unintended side effects. The following case report details the early clinical signs, diagnostic approach, surgical correction, prognosis, pathophysiology, and follow up considerations of a unilateral GTCT, in the lens of current and past literature.

History & Presentation

  Bling (KM Jazz It Up) is an approximately 11-year-old quarter horse mare primarily used for western pleasure and trail riding. She presented to Mississippi State University College of Veterinary Medicine Equine Services on May 27th, 2020 for a modified standing right flank hand-assisted laparoscopic unilateral ovariectomy to remove a suspect GTCT. Prior to presentation Bling had a prolonged history of stallion-like behavior coupled with unusual and irregular cyclicity. The referring veterinarian had rectally palpated a large mass on the right ovary prior to referral.

  Upon presentation Bling was bright, alert, and responsive. She weighed approximately 1152 lbs. and had an appropriate BCS of 5/9. Bling's vital parameters were within normal limits with a respiratory rate of 8 breaths per minute (range 8-12 breaths per minute), temperature of 99.7 degrees Fahrenheit (range 99-101.5 degrees Fahrenheit), and a slightly elevated heart rate at 56 beats per minute (range 24-48 beats per minute) presumably due to the recent trailer ride.

Cardiopulmonary auscultation revealed no crackles, wheezes, or irregular heart sounds. Her mucus membranes were pink, moist, and with a normal capillary refill time of <2 seconds. Gastrointestinal motility was normal in all four quadrants and no digital pulses were detected in all four limbs. She was diagnosed with a grade 2/5 lameness in the right hind limb becoming slightly more pronounced when circled, but this was considered separate from the primary complaint. The remainder of her physical exam was within normal limits.

Diagnostic Approach and Differential Diagnosis

In the case of a GTCT, the clinical picture can at times be misleading, and requires a most astute veterinarian. Often the only clinical sign may be a history of failure to conceive, which in turn can lead to a delayed diagnosis in horses not routinely bred[7,9]. Additional clinical clues such as erratic stallion-like behavior, a painful abdomen, nymphomania, and increased aggression may also manifest[2,5,6,7,9]. Less common clinical signs include mammary gland enlargement, exercise intolerance, and a stiffened gait[9]. At this time there is not believed to be a breed predisposition and a genetic component has not yet been identified. While this disease is most often unilateral in presentation, bilateral cases have been reported[2] and there is no apparent site predilection between right or left ovary.

Following a thorough physical evaluation, a rectal examination and transrectal ultrasound are the appropriate next steps as these can help to identify the affected ovary, rule out other causes of infertility (viral, bacterial, fungal, mechanical, obstructive, etc.), and gauge the severity of the presumptive neoplasm. Other differentials to consider include teratoma, cystadenoma, cystadenocarcinoma, cysts, hematoma, lymphoma, dysgerminoma, thecoma, leiomyoma, fibroleiomyoma, and ovarian abscess[2,7,9]. Hasty ultrasound examination can confuse persistent

anovulatory follicles or multiple *corpus lutea* for a multiloculate silhouette suggestive of a GTCT[7,9], therefore, rectal palpation and ultrasound should be used in conjunction. Rectal palpation will often reveal an appreciably enlarged ovary with a multilobulated surface or large outer capsule. Rectal ultrasound findings are most often diagnosed by a honeycomb or multiloculated anechoic mass on the affected ovary [7,9]. It should be emphasized that on occasion, ovarian enlargement may not be present, complicating the diagnosis[9]. Preliminary research has explored the efficacy and safety of ovarian biopsy for diagnosis of GTCT[1] but is not currently utilized at this time. Should these findings further support the diagnosis of a GTCT, a fresh blood sample should then be submitted for a hormone panel. The hormone panel determines blood levels for anti-Mullerian hormone, testosterone, and inhibin. This hormone panel can be diagnostic for the presence of granulosa and theca cells, but definitive diagnosis must be confirmed on histopathologic evaluation[7,9]

Upon arrival, the mare was sedated with intravenous butorphanol (0.3 mLs) and dexmedetomidine (0.3 mLs) given with buscopan to relax the rectum prior to palpation. On rectal examination, and confirmed with ultrasound, a large multilobulated fluid-filled mass (approximately 20cm in diameter) on the right ovary was detected. On rectal examination it was also noted that the cecum was displaced cranially by the right ovary eliminating the cecum as an obstruction for a right flank laparotomy. A hormone panel had been previously submitted on May 5th, 2020 and returned with elevated inhibin (>2-100 pg/ml), testosterone (>20-45 pg/ml), and anti-Mullerian hormone (>0.1-6.9 ng/ml). A low progesterone level was also detected suggesting the absence of luteal tissue (<0.5 ng/ml). With all these clinical and diagnostic findings, a presumptive diagnosis of a GTCT was made.

Pathophysiology

Granulosa Theca Cell tumors are commonly benign unilateral neoplasms that can present in multiple species with varying prevalence. In the equine species, these are the most common ovarian tumor making up approximately >2.5% of all equine tumors[9] and commonly seen in older mares >5 years of age with the average age being around 11 years[7,9]. It has, however, been reported in all age groups[7,9]. These tumors are made up of a myriad of granulosa, theca cells, and occasionally Leydig-like cells. These specialized stromal cells are important for hormone secretion and reproductive cyclicity[4,6,7,11]. Hormonally these tumors dysregulate normal hypothalamic-pituitary-axis (HPA) function and lead to an increased output of Anti Mullerian hormone, inhibin, testosterone, and decreased progesterone. This cocktail of hormones manifests clinically in periods of prolonged anestrus, intermittent, extended, or irregular estrus, and stallion-like behavior in more severe cases[4,6,7,11]. Physiologically, inhibin produced by the GTCT inhibits production of FSH (follicle stimulating hormone) which in turn prevents growth of new follicles. Lack of follicular growth is consistent with anestrus and further reproductive hormone imbalance. Testosterone production from the Leydig-like cells is thought to be the cause of stallion-like behavior and increased aggression[7,9]. Commonly the opposite ovary will atrophy or lose functional capacity hypothesized to be a result of the suppressive effect of the GTCT and downstream HPA axis disruption [4,7] but has not yet been experimentally explained[11]. In most cases removal of the neoplastic ovary will lead to a return to function of the contralateral ovary. There is some discourse in the literature as to whether delayed return to estrus may be correlated with increased chronicity and size of the tumor [9].

Histologically, these tumors are a blending of poorly organized granulosa cells and theca cells. Occasionally, in higher testosterone producing tumors, there are also Leydig-type cells

intermixed[4,7]. Classified as sex-chord stromal tumors, these are specialized ovarian tumors that can even develop from any ovarian tissue and even remnant tissue in the case of an incomplete ovariectomy[7]. A definitive diagnosis can be made histologically if Call-Exner bodies, aggregates of granulosa cells surrounding bodies of proteinaceous fluid, are observed[7,9]. Histologically, expect to see few criteria of malignancy such as anisocytosis, anisokaryosis, and a low mitotic index[7]. These are often fluid filled tumors and therefore it is common to see a pseudo-alveolar pattern or large cystic areas surrounded by granulosa cells[7]. Fibrin, collagen, hemorrhage, and bands of stroma arranged sporadically or in a tubular pattern are also commonly reported findings[7].

Treatment & Management

Treatment is generally warranted as GTCT may continue to grow until the point of rupture leading to a hemoabdomen or peritonitis, can cause abdominal pain, may adhere to other abdominal tissues, and potentially hinder the performance of a horse used in competition[6,8,9]. As emphasized earlier, the abnormal behavior pattern can also put handlers and riders at a greater risk for injury. In valuable broodmares this disease can lead to prolonged periods of anestrus and irregular breeding cycles resulting in considerable economic loss and frustration. Currently, the preferred treatment is a unilateral ovariectomy of the affected ovary leaving the remaining ovary intact[5-9]. Surgical excision may be performed laparoscopically[5,6,8,9,10] in a standing flank procedure, rarely via colpotomy, or if the tumor is too large it may be removed via a ventral midline incision under general anesthesia[5]. A ventral midline approach allows for better visualization of the tumor but also carries more risk due to the need for general anesthesia and the more invasive nature of the procedure[5,6,8,9,10]. A laparoscopic approach has many advantages

(no need for general anesthesia, smaller incision site, and less associated surgical complications) but choice of approach often comes down to surgeon preference and resources available. Surgical excision is well documented and is the only known curative treatment at this time [5-10]. General surgical considerations should be monitored such as incisional dehiscence, local or systemic infection, decreased water consumption, and reduced overall fecal output. Complications include hemorrhage from the ovarian pedicle, post-operative abdominal pain or colic, seroma formation, incisional infection, adhesion of the uterine horn to the surgery site, laminitis, and varying degrees of colitis [5,6,8,9,10].

Post-operative management is crucial in preventing these complications. General recommendations are 10-14 days of stall rest and a gradual return to exercise[5-10]. This greatly reduces the chance for dehiscence of the internal and external sutures and may reduce the risk of post-operative colic. Judicious use of antimicrobials and NSAIDs should be continued short-term following surgery to reduce risk of infection and inflammation at the surgical site [5-10].

Prognosis in these cases is promising especially with early detection and smaller tumor size. Additionally, the reproductive prognosis is encouraging as these mares are often able to be bred back in a matter of months and successfully carry their foal to term[4-11]. Early detection of this tumor reduces the chances of surgical complications as the tumor is a more manageable size, but overall, this is a favorable procedure with a promising outcome at all stages. Recurrence of this tumor on the contralateral ovary is a possibility but has been rarely reported[2,9].

In preparation for surgery, Bling was administered intravenous Gentamicin (6.6mg/kg) 35 ml q 24 to target gram negative bacteria coupled with intravenous Penicillin G potassium (22,000 IU/kg) 23 mL IV q6hr for efficacy against gram positive bacteria both perioperatively and intraoperatively. Together these two antimicrobials offer a broad spectrum of antimicrobial

coverage. Flunixin meglumine 1.1mg/kg 11ml IV q12h was also administer preoperatively and post-operatively to reduce inflammatory side effects from the surgery. On May 27th, 2020 the mare underwent a modified right standing hand assisted laparoscopic ovariectomy. She was fasted for 24 hours prior to surgery. An intravenous catheter was placed in the right jugular vein and the patient was secured in the stocks. Sedation was achieved with a combination of 3mg of dexmedetomidine and 10mg of butorphanol intravenously on a continuous rate infusion (CRI) and maintained throughout the procedure. The right paralumbar fossa was clipped with wide margins and then aseptically prepared for 10 minutes with 4% chlorhexidine soap and alcohol. The patient was draped with paper drapes secured with backhaus towel clamps. A square opening was made through the drape at the level of the paralumbar fossa for the proposed surgery site with mayo scissors.

An inverted L line block was performed injecting small amounts of lidocaine subcutaneously from the 5th lumbar vertebrae proximally to the last rib and then ventrally along the last rib. This provided better intraoperative analgesia for the procedure. Subsequently, a 10 cm vertical incision was made in the paralumbar fossa with a #10 scalpel blade. The incision was continued through the subcutaneous tissue, facia, and external abdominal oblique. A modified grid approach using manual blunt dissection was performed to divide the internal and transverse abdominal oblique muscles providing a window to the peritoneum. The peritoneum was bluntly penetrated using hemostats and the opening was enlarged to accommodate the surgeon's hand. The right ovary was visualized and grasped firmly using Babcock 10 mm grasping forceps. Finochietto rib retractors were placed to maintain a patent incision site and provide better access. Once visualized and stabilized, a spinal needle attached to a 60mL syringe was used to inject approximately 20ml of bupivacaine into the ovary. Next a second spinal needle and syringe were

placed to begin to aspirate the fluid filled neoplastic parenchyma. In total 750 mLs of yellow to serosanguinous transudative fluid was aspirated prior to removal of the ovary. Once reduced in size, the ovarian pedicle was visualized, and bupivacaine was injected into the ovarian pedicle to provide further analgesia. While still holding the right ovary in one hand, a vessel sealing device (Ligasure) was introduced through the flank incision and used to transect the mesovarium while also maintaining hemostasis. Once further removed an intraabdominal staple gun (TA 90 Stapler) was used to ensure the pedicle was completely clamped off. In total 18 staples were placed in two parallel strips. The ovary was then surgically dissected distal to the staples and cauterized tissue and then manually removed from the flank incision. The ovary was placed in a specimen container for pathologic examination. The mesovarium and the remaining ovarian pedicle were examined for any hemorrhage and none was noted. The omentum was then thoroughly lavaged with irrigation saline and Gentamicin prior to being replaced in the abdomen. The transverse fascia and internal oblique muscle were closed with cruciate pattern using #2 Vicryl. The external oblique and subcutaneous tissues were then sutured closed in a simple continuous pattern using 2-0 PDS on a taper needle. Finally, the skin was opposed with a transdermal, and vertical mattress pattern. No staples were used externally.

Post-operatively the mare was monitored closely for signs of pyrexia, inappetence, pain, and fecal incontinence. Antibiotics and NSAIDs were continued a week after surgery, and she was restricted to stall confinement. A cold ice compress was applied to the incision site daily to help reduce inflammation around the surgical site. The mare was successfully discharged on June 8th, 2020.

Case Outcome

The surgery was uneventful, and her recovery was without complications. Two days post operation she had a mild fever and ancillary bloodwork was performed and was consistent with a stress leukogram (neutrophilia, lymphopenia, and eosinopenia). Outside of this incidence her recovery was uneventful. The ovary was submitted in its entirety for histopathology and was definitively diagnosed as a GTCT. The diagnostic pathologist described the submitted mass as grossly multiloculated and multi-cystic with highly variable diameters and an alveolar-like pattern. Histologically the mass was lined by neoplastic epithelial cells interlaced with occasional bands of collagenous and fibrovascular stroma. The cells varied between either 1 cell thickness deep or up to 30 cells in thickness surrounding the larger cysts, but rarely formed cellular sheets. There was mild anisocytosis and anisokaryosis with a mitotic rate of 4 in 10 high powered fields all suggestive of a relatively benign tumor. Interestingly, there were only a few examples of Call-Exner bodies. Within the mass there were multifocal areas of fibrin, edema, and hemorrhage. These findings are highly indicative of a GTCT and the reporting pathologist confirmed the diagnosis. Now almost 1-year post-op the mare has progressed wonderfully. She no longer competes but has had no more incidences of stallion like behavior, irregular cyclicity, or evidence of recurrence. In Spring of 2021 the mare was successfully bred with two embryos collected and placed in two separate recipient mares.

Discussion

This classic case highlights many important aspects of clinical medicine as well as a collaborative approach to diagnosis and treatment. The key to this case lies in early detection and consistent dialogue with the client. While these tumors carry a generally good prognosis, early detection reduces the surgical risks and allows for a range of surgical approaches as the tumor is

often smaller and more manageable. This case illustrates the importance of a thorough anamnesis (history taking) as the more fastidious veterinarian may be able to detect the early warning signs of this neoplasia sooner. As the research literature and case reports continue to be published, it is entirely possible the incidence of bilateral GTCT as well as recurrent GTCT on the contralateral ovary may also increase in prevalence. Therefore, if a mare is not intended for breeding purposes, it may be of benefit to perform a complete ovariohysterectomy. Lastly this case is a reminder of the age-old adage, "neoplasia does what neoplasia wants", therefore a flexible diagnostic mindset is essential as often only one or few of the previously described clinical signs may manifest. Clinical pattern recognition is an important skill to refine, but there is always an exception to every rule.

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