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Equine nonneoplastic abnormal ovary in a draft mare with high serum anti-Müllerian hormone: a case study

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We performed a standing hand-assisted laparoscopic ovariectomy in a draft mare that presented with high serum anti-Müllerian hormone (AMH) level and had an enlarged single cystic ovary. Histopathological examination revealed no tumor cell proliferation in the ovary, but the presence of a large ovarian cyst was confirmed. In the diagnosis of abnormal ovaries in mares, a comprehensive assessment should be performed, including the monitoring of ovarian morphology and biomarkers over time, to determine the disease prognosis and treatment plan. The case of this mare with a nonneoplastic abnormal ovary and increased serum AMH level was rare. We suggest that standing hand-assisted laparoscopic ovariectomy is useful for the removal of large ovaries in draft mares.

Key words: *anti-Müllerian hormone, draft mare, laparoscopic ovariectomy, ovarian cyst*

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Ovarian enlargement in mares can be caused by nonneoplastic abnormalities [17], including hematomas [5], ovarian abscesses [15], and neoplastic diseases such as granulosa cell tumors (GCTs) [4]. Among ovarian tumors, GCT is the most common in mares, whereas other types, such as teratomas and cystadenomas, are rare [4]. GCTs can occur in mares of all ages, from young to old. Most GCTs occur unilaterally and are rarely malignant. Affected mares present with symptoms such as anestrus, persistent estrus, and male behavior and are infertile in most cases. To our knowledge, there is currently no effective medical treatment for GCT, which necessitates the surgical removal of the affected ovary [4]. For GCT diagnosis, in addition to clinical signs and rectal examination, ultrasound may be performed, and blood inhibin and testosterone levels may be measured. However, owing to the lack of consistency among cases and the difficulty in differentiating GCT from similar diseases, such as ovarian hematoma and abscesses, making a definitive diagnosis in clinical settings is challenging [16]. Recently, anti-Müllerian hormone (AMH) has attracted scientific attention as a potential biomarker for GCT. AMH is expressed by granulosa cells in the ovary, and mares with GCT have increased serum AMH levels [1, 3]. Increased serum AMH level has a sensitivity of 97.7% in the diagnosis of GCT [2]. In our case, we performed

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a standing laparoscopic ovariectomy in a draft mare with a unilateral single cystic large ovary and a high serum AMH level. Approximately 5 months after surgery, the mare mated, conceived, and eventually gave birth. This case report presents an overview of our treatment plan in combination with clinical examination and laparoscopy for an ovarian disease in a draft mare with the hope that it will aid in the diagnosis of abnormal ovaries in clinical settings.

During a genital examination of a primiparous draft mare (age, 9 years; body weight [BW], 726 kg) on day 9 after foaling, a large right ovary, which was approximately the size of a basketball, and a normal-sized left ovary were observed. The uterus had sunk deeply into the bottom of the abdominal cavity. An ultrasound examination revealed the presence of a single cyst with a diameter of >25 cm in the right ovary but no clear structures in the left ovary (Fig. 1). Hysteroscopy, endometrial biopsy, bacterial cultures, and cytology were then performed, but no abnormal findings were observed. Serum samples were submitted to the laboratory of the Hidaka Training and Research Center, Japan Racing Association, for measurement of the AMH level. The serum AMH level was 33.47 ng/m*l*, which was higher than the reference range (0.1–6.9 ng/m*l*) [16]; therefore, a GCT was suspected. Thereafter, the blood AMH level remained high, but cyclic and clear signs of estrus were confirmed in the contralateral (left) ovary. For this reason, the mare was mated, and ovulation was confirmed. In total, the mare mated three times in the season but did not conceive after any of the mating events. After obtaining consent from the owner, laparoscopy-assisted ovariectomy was performed on the right ovary. Before the surgery, the mare was subjected to fasting for 24 hr and was retained in the treatment stock with standing sedation using intravenous medetomidine (3 μg/kg BW; Dorbene, Kyoritsu Seiyaku, Tokyo, Japan) and butorphanol (0.1 mg/kg BW; Vetorphale, Meiji Seika Pharma, Tokyo, Japan) injections. The location of the blood vessels running through the right flank was confirmed by ultrasound examination (LOGIQ e Premium, GE Healthcare, Tokyo, Japan). In addition, intravenous flunixin meglumine (1 mg/kg BW; Forvet, MSD Animal Health, Tokyo, Japan) and intramuscular penicillin and streptomycin (penicillin, 10,000 IU/kg BW; streptomycin, 12.5 mg/kg BW; Mycillin, Tamura Seiyaku Corp., Saitama, Japan) were administered before surgery. Subcutaneous 2% lidocaine solution (Xylocaine, AstraZeneca, Osaka, Japan) was used to anesthetize the incision sites on the skin. A cannula was then inserted into the central part of the right flank, and a pneumoperitoneum was produced using CO_2 at an intra-abdominal pressure of approximately 10 mmHg. A cannula was inserted into the last intercostal space and then used as the laparoscopic port, and a third cannula was inserted into the ventral side of the flank. Using a laparoscope (Stryker Japan K.K., Tokyo, Japan), the right ovary was observed to have significantly descended into the deep site of the abdominal cavity, with only the dorsal part of the ovary visualized. Local anesthetic (2% lidocaine solution) was injected into the mesovarium, and approximately 7.5 *l* of ovarian fluid was aspirated to reduce the size of the ovary. A part of the mesovarium was then separated using the LigaSure Vessel Sealing System (LigaSure, Covidien Japan, Tokyo, Japan). A hand was inserted through the 12-cm incision site on the right flank (hand assisted) [9], and the wide area of adhesion between the large ovary and mesovarium was removed manually. The ovary was then separated from the mesovarium and uterus with LigaSure and scissors. The large ovary was put into a sterilization pouch and then cut into tissue masses with a large quantity of fluid [6]. The sterile bag and excised ovary were then removed from the mare. To close the incision site, three-layer suturing was performed on the muscular layer using absorbable

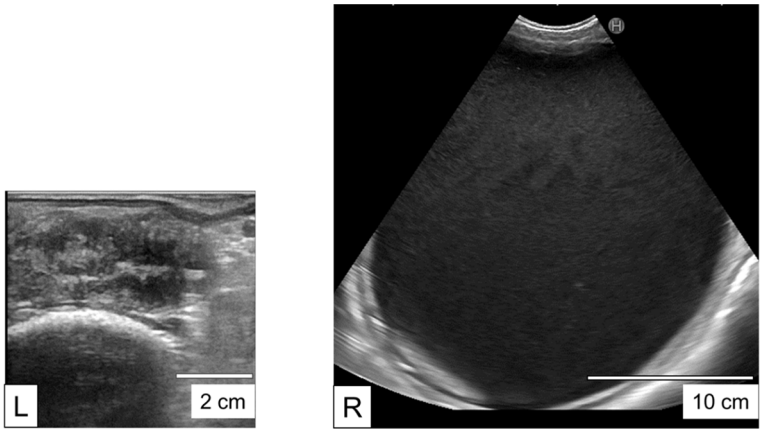

Fig. 1. Ovarian findings during the rectal examination of the mare on day 9 after foaling.

suture, and the skin was subsequently sutured. To close the cannula insertion sites, only the skin was sutured. The total duration of the surgery was approximately 4 hr. The total weight of the excised ovary, including the ovarian fluid, was approximately 15 kg. No postoperative complications were encountered. Intravenous flunixin and intramuscular mycillin were administered once daily for 3 days after the surgery. The postoperative course was uneventful, and the mare was discharged after 3 days of hospitalization. After the ovariectomy, the serum AMH level decreased to 0.31 ng/m*l* (Fig. 2). For pathological analysis, some parts of the excised ovary were fixed in 10% formalin solution and embedded into paraffin. The paraffin block was sectioned into 4-μm slices using a REM-710/SB gliding microtome (AS ONE Corp., Osaka, Japan). For histopathological evaluation, tissue sections were stained with hematoxylin and eosin. Histopathological examination of the excised ovary revealed that the lumen of the cyst was filled with inflammatory cell debris and exfoliated epithelial cells. In the examined area, the epithelial lining was unclear (Fig. 3A). The cyst wall was thick. It contained a smooth muscle layer, collagenous tissue, and few glandular structures lined with cuboidal epithelium. The epithelial cells did not show cellular atypia, and there were no neoplastic structures. The histological findings suggested that the cyst was an ovarian cyst; however, precise identification could not be achieved. For immunohistochemical analysis, sections were incubated with an anti-AMH goat antibody (1:500; sc-6886, Santa Cruz Biotechnology Inc., Dallas, TX, U.S.A.). For color development, a Vector® NovaRED™ Peroxidase (HRP) Substrate Kit (SK-4800, Vector Laboratories, Inc., Burlingame, CA, U.S.A.) was used according to the manufacturer's guideline. A tissue section from an equine ovary with GCT was used as a positive control, and a tissue section incubated with diluent buffer instead of primary antibody was used as a negative control. Immunostaining of AMH showed negative staining in the ovarian cyst wall (Fig. 3B), whereas the positive control showed positive staining of AMH in granulosa cells (Fig. 3D). The mare mated naturally 5 months after the surgery, conceived, and gave birth in the following year.

In the diagnosis of abnormal ovaries in mares, the anatomical and physiological changes of the ovaries vary greatly, and there is no consistency among cases, which poses diagnostic challenges [16]. In the present case, the mare presented with high serum AMH levels immediately after delivery, and a large cyst was found in the right ovary. Although cyclic estrus with ovulation was confirmed several times in the contralateral ovary, the mare did not conceive. The serum AMH level has recently been proposed as a useful biomarker for the diagnosis of GCT in abnormal ovaries [1, 3]. However, in our case, a histopathological examination refuted the diagnosis of GCT. In addition, the mare showed normal estrus cycling despite the high AMH level; therefore, we inferred that granulosa cells did not secrete excessive inhibin, which is one of the biomarkers of GCT. Furthermore, after ovariectomy, the serum AMH level in the mare decreased rapidly, suggesting that the source of the excess AMH was the removed abnormal ovary. The function of abnormal ovaries is not uniform; in some cases, even in mares diagnosed with GCT, the blood AMH level repeatedly fluctuates within the normal range [16]; in other cases, however, mares have a functional contralateral ovary [11]. A previous study reported that GCT healed

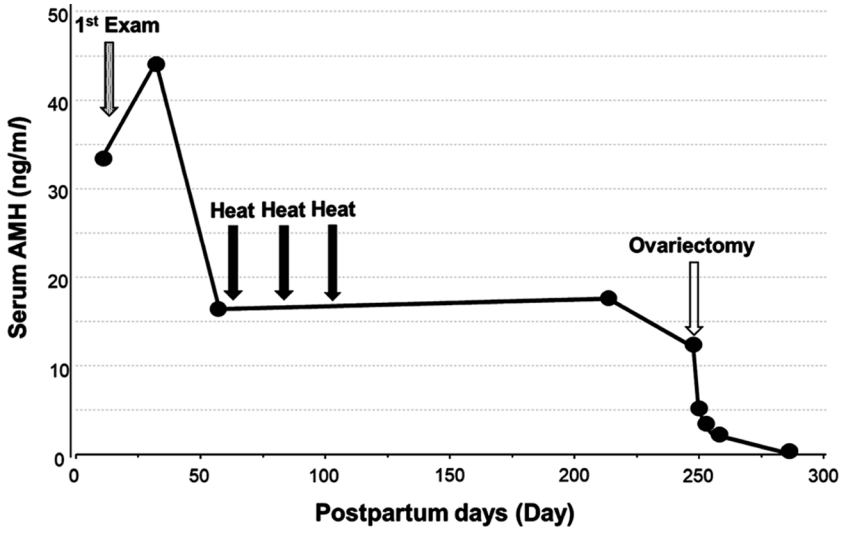

Fig. 2. Transition of the serum anti-Müllerian hormone (AMH) level and events from delivery to ovariectomy in the mare.

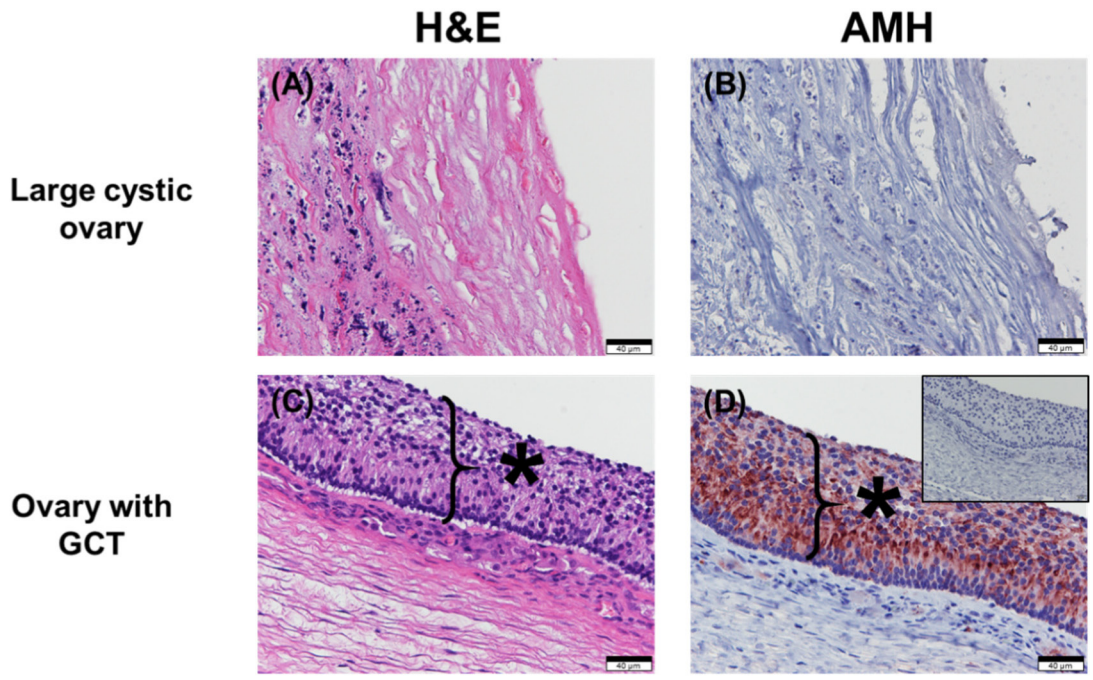

Fig. 3. Histopathology and immunostaining of anti-Müllerian hormone (AMH) in the large cystic ovary and control sample. (A) The large ovarian cyst wall was thick and contained collagenous tissue. Hematoxylin and eosin (H&E) staining; bar=40 μm. (B) AMH was not expressed in the cyst wall of the large cystic ovary. Immunohistochemical (IHC) staining; bar=40 μm. (C) A tissue section of an ovary with granulosa cell tumor (GCT; control) revealed granulosa cells (*) in follicle-like structures. H&E staining; bar=40 μm. (D) AMH was produced in granulosa cells (*) of the positive control sample (ovary with GCT); brown color indicates positive staining. IHC staining: bar=40 μm. Insert: negative control slide showed no staining of AMH.

naturally without ovariectomy [12]. The literature thus suggests the need for continuous observation of ovarian morphology and the monitoring of biomarkers to diagnose abnormal ovaries. Murase *et al*. [14] reported increased serum AMH levels in two mares with transient ovary enlargement, which was not secondary to GCT. Notably, a histopathological examination was not performed for either mare [14]. In addition, Renaudin *et al*. [16] reported two cases of mares with abnormal ovaries and increased serum AMH levels, although the diagnosis of GCT was excluded after a postovariectomy histopathological examination. The pathophysiology was similar in all these cases. In our case, the serum AMH level went from 33.47 ng/m*l* on postpartum day 9 to 43.94 ng/m*l* on postpartum day 30. Thereafter, it decreased to 16.41 ng/m*l* on postpartum day 60, and estrus returned (Fig. 2). This suggests that ovarian function and AMH secretory ability were altered between postpartum days 30 and 60. The serum AMH level was 12.28 ng/m*l* at the time of ovariectomy (Fig. 2); the immunostaining of AMH, however, showed negative staining of the ovarian cyst wall. The excised ovary was quite large, so the lining cells of the cystic ovary could have been compressed and degenerated. We tried immunostaining the remaining cystic wall but failed to evaluate the AMH-producing cells. Thus, we speculate that the serum might have derived AMH from the remarkably enlarged ovary because the systemic AMH level dropped immediately after ovariectomy. Further studies on other cases of abnormal equine ovaries based on the measurement of AMH levels are warranted.

In humans, AMH is an important regulator of follicle maturation; abnormally increased AMH negatively affects reproductive functions through androgen overproduction due to the expression of aromatase and decreased follicle-stimulating hormone sensitivity in ovarian follicles [8]. Similar to the reports in humans, the increased serum AMH level in our case might have negatively affected the reproductive performance of the mare. In addition, the large ovary was adhered to a wide area of the mesovarium, and the uterus was excessively pulled together, with the enlarged ovary toward the bottom of the abdominal cavity. Therefore, physical disorder of the ovary and uterus might have also been a reason for infertility.

Equine laparoscopic surgery has previously been applied to ovariectomy, cryptorchidectomy, and intestinal surgery [10]. In our case, we performed a standing laparoscopic ovariectomy on a draft mare. This procedure is effective,

safe, and minimally invasive [9]. To our knowledge, there are no previous reports of a successful standing laparoscopic ovariectomy in a draft mare, although there have been successful reports in other breeds, such as thoroughbreds and warmbloods [9]. Laparoscopic ovariectomy in the supine position should be performed in the Trendelenburg position to facilitate access to ovaries, and general anesthesia is necessary [10]. From the perspective of respiratory management risk reduction under anesthesia in large mares, such as draft mares [7, 13], standing laparoscopic ovariectomy carries a decreased risk. Furthermore, in the present case, we were able to remove the large ovary with a minimal incision by chopping it into small pieces in a sterilization pouch. Our results suggest that standing laparoscopic ovariectomy is a minimally invasive and safe procedure for draft mares.

The abnormal ovary of the mare in our case was confirmed to contain an ovarian cyst. Although some mares might have increased serum AMH levels in association with nonneoplastic abnormal ovaries, this is considered a very rare occurrence. In the diagnosis of abnormal ovaries in mares, it is important to determine the treatment plan and prognosis based on a comprehensive assessment, including the monitoring of ovarian morphological changes and biomarkers over time, and the confirmation of periodic estrus. Furthermore, standing laparoscopic-assisted ovariectomy may be useful for the removal of enlarged ovaries in draft mares with a high risk of anesthesia-related complications.

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