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Comparative Evaluation of 2-Port Laparoscopic Ovariectomy Using LigaSure versus Standard 3-Port Laparoscopic Ovariectomy with a Bipolar Electrode in Mares

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Abstract
Ensuring fast and efficient hemostasis is crucial for achieving optimal outcomes in laparoscopic ovariectomy surgery. This study compared the clinical outcomes of standing laparoscopic ovariectomy for medium-sized granulosa cell tumors (≤15 cm in size) using a 2-port LigaSure versus a 3-port bipolar electrode, focusing on operating time, mean blood loss, intraoperative and postoperative complications, and the duration of the prospective hospital stay. Twelve mares were divided into two groups: six underwent standing laparoscopic ovariectomy with LigaSure through a 2-port approach, while the remaining six underwent the standard 3-port procedure with the bipolar electrode. Our findings demonstrated that 2-port laparoscopic ovariectomy using LigaSure was not only technically feasible and safe but also offered several advantages, including shorter operating times, simplified procedures, decreased postoperative analgesic requirements, and improved cosmetic appearance of surgical wounds. Moreover, this technique proved to be a reliable method for achieving hemostasis of the mesovarium while also being technically straightforward, time-saving, and cost-effective. Overall, our study suggests that 2-port laparoscopic ovariectomy with LigaSure is a promising alternative to the standard 3-port approach. This approach not only benefits patients by potentially reducing postoperative discomfort and enhancing recovery but also provides advantages for surgeons in terms of efficiency and resource utilization.

Keywords
Laparoscopic; Ligasure; minimally invasive; mare; ovariectomy; granulosa cell tumor

1. Introduction
Granulosa cell tumors (GCTs) are the most common ovarian tumors found in mares, accounting for more than 85% of reproductive neoplasms and approximately 2.5% of all neoplasms in horses [1]. These tumors originate from the follicular granulosa, with a subset known as granulosa-theca cell tumors (GTTs) also containing a distinct theca-derived component [2]. This distinction can be identified through histopathological examination and is functionally characterized by the capacity for androgen secretion [3].

The majority of cases of GCT are unilateral, and concurrent atrophy of the contralateral ovary provides support for the presumptive diagnosis of GCT [4]. Various surgical techniques have been utilized for the removal of ovaries, particularly those affected by granulosa thecal cell tumors, in mares [5]. These methods include the ventral midline approach [5], the flank approach (potentially involving laparoscopy) [6,7], and colpotomy via the vaginal wall [8]. The choice of approach is influenced by factors such as ovarian size, surgeon preference, and the overall health condition of the animal [9,10], while smaller and medium ovaries are extracted through the flank

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using either an open, laparoscopic, or laparoscopic-assisted approach [2,9].

Standing laparoscopic ovariectomy has emerged as a more frequently employed technique for ovarian removal in mares [11–13]. Compared to traditional laparotomy-based procedures, laparoscopic methods offer several advantages, such as reduced complications due to better visualization, less invasiveness, efficient hemostasis, shorter recovery times, fewer postoperative complications, and secure vessel ligation within the mesovarium [14–21]. However, a significant challenge with laparoscopic ovariectomy is determining the best approach for ligating the ovarian pedicle and achieving hemostasis [22,23]. Consequently, various techniques have been utilized, including stapling instruments [23,24], laser methods [25], ligature application [23], vascular clips [26], and electrocoagulation [27]. However, these hemostatic techniques often prove to be time-consuming and challenging to apply and may lead to intraoperative hemorrhage [28]. The use of precise techniques and instruments is crucial for achieving optimal outcomes in laparoscopic surgery [29].

Advances in energy-based vessel sealing technologies, including bipolar sealing devices, ultrasonic devices, and nanotechnology-based devices, have expanded options for achieving hemostasis [30,31]. Among these, LigaSure stands out for its superior burst pressure and fast sealing time, effectively sealing vessels up to 7 mm in diameter while minimizing thermal spread [31–33].

LigaSure has been used with a high success rate in equine surgery [34,35]. Furthermore, employing reduced-port laparoscopic surgery optimizes the surgical procedure, minimizes instrument interference, reduces surgical incisions, and promotes faster postoperative recovery [20,36,37].

This study aimed to (1) evaluate the clinical application and outcomes of a 2-port laparoscopic ovariectomy in mares using LigaSure compared to a standard 3-port laparoscopic ovariectomy and (2) conduct a comprehensive histological evaluation of these soft masses to enhance the understanding of their nature and characteristics.

2. Materials and Methods

The present study adhered to ethical protocols and received approval from the Faculty of Veterinary Medicine at Damanhour University (Ref. No. A02/2023). Surgical procedures were performed in the Animal Panorama Center of Excellence, Faculty of Veterinary Medicine, Damanhour University. Between October 2018 and April 2022, a total of twelve mares of the local breed were included in the study and underwent elective standing unilateral laparoscopic ovariectomy. These mares exhibited unilateral ovarian pathologies characterized by soft tissue masses, as diagnosed by ultrasound examination **(Figure 1)**.

The primary objective of this investigation was to evaluate the technical feasibility, safety, and benefits of performing a 2-port laparoscopic ovariectomy utilizing LigaSure in comparison to the conventional 3-port procedure. A histological analysis of the ovarian masses was conducted. Technical feasibility was defined as the ability to easily conduct laparoscopic ovariectomy using a single visual port for the laparoscope and another instrumental port for the LigaSure device or two ports for the bipolar handle and grasping forceps. Safety was evaluated based on the absence of major complications, such as bleeding and damage to visceral organs. The outcomes of the procedure were evaluated by considering various factors, including operative time, ease of the procedure, postoperative analgesia according to the visual analog scale, duration of hospital stay, and cosmetic appearance of surgical wounds. The operative time was measured from the initial incision to the completion of wound closure. The ease of the procedure was scored by the operating surgeon on a scale of 0-3, where 0 indicated no exertion by the surgeon, 1 indicated an easy procedure, 2 referred to difficulty, and 3 suggested extreme difficulty requiring an additional port for completion. The scoring parameters for ease of assessment were based on observations of the laparoscopic field, accessibility of organs, tissue handling, and the number of operative failures. Postoperative pain was assessed using the visual analog scale (VAS), as per standard practice [38], where 0 on the visual analog scale (VAS) indicated the absence of pain, and 10 indicated severe pain. Postoperative cosmetic outcomes were evaluated by another independent surgeon 30 days after the procedure using a scale ranging from 1 (worst: the wound is infected and inflamed with exudation) to 2 (average: the wound is inflamed or swollen without infection or exudation) to 3 (best: the wound is clean, has completely healed, and has no inflammation or infection). A follow-up assessment was conducted by the operating surgeon 30 days postoperatively through a telephone questionnaire to gather information on any complications that arose after discharge, pain scores, and the cosmetic scale.

2.1. Preoperative Measures

Prior to surgery, routine preoperative measurements were taken, which included a comprehensive physical examination, gynecological examination per rectum, transrectal ultrasound (Sonoscape E2 vet Expert, China), intravenous catheter placement into the jugular, and complete bloodwork. Food was withheld for 24 hours before the surgery, while water intake was not restricted. Two hours before the procedure, the mares were administered Procaine penicillin (22000 U/kg) im and flunixin meglumine (1.1 mg/kg) iv. Immediately before the surgery, the mares were positioned in stock and given detomidine hydrochloride (0.002-0.004 mg/kg IV) as a bolus, with repeat doses as necessary, to provide sedation and analgesia throughout the surgical procedure. The relevant paralumbar fossae were then prepared and draped in an aseptic manner. An inverted-L block was performed using lidocaine, with 50 mL of a 10 mg/mL solution (Lidocaine, Hospira Inc., USA) injected into each fossa.

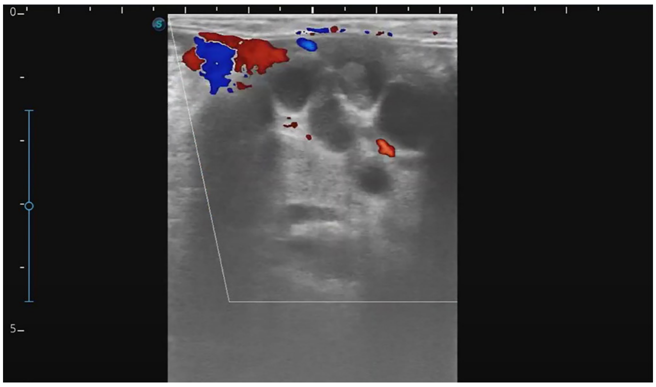

Figure 1: Transrectal color Doppler ultrasound of the ovary revealed a multicystic appearance and active vascularization.

2.2. Two-Port Laparoscopic Ovariectomy (Figure 2)

The 2-port technique was employed on a total of six mares (n = 6). To initiate the technique, one port was inserted into the laparoscope, while another instrument port was placed in the flank. The laparoscope portal, measuring 10 mm, was positioned 3 cm caudally to the last rib and ventrally to the ventral border of the coaxial tuberosity. An approximately 1 cm incision was made in the skin and through the abdominal wall, and a trocar-cannula set of 10 mm (AED, USA) was advanced through the abdominal wall into the peritoneal cavity. A 10 mm 0-degree laparoscope was then introduced (Karl Storz, Germany).

The second operative port, also measuring 10 mm, was placed caudal and ventrally approximately 6-8 cm from the first port. A skin-stab incision of approximately 1 cm was made, and a trocar-cannula unit of 10 mm (AED, USA) was pushed through into the peritoneal cavity after dissection of the abdominal muscles. CO_2 was used to insufflate the abdomen at a rate of 6 L/min, achieving a pressure of 12 mm/Hg, which exposed the ovary and created the operative field. Subsequently, an 18-gauge 30 cm histological needle (HS Medical, China) was inserted through the secondary instrumental port to induce local anesthesia of the ovary and mesovarium.

Infiltration anesthesia of the ovarian pedicle was performed using a 20 mL solution of 1% lidocaine (Lidocaine, Hospira Inc., USA), after which the needle was removed. Two minutes later, a 10 mm-37 cm LigaSure (Covidien, USA) was applied through the secondary port across the mesovarium. The instrument was closed, triggering the cautery to seal the tissue. The second trigger was used to cut the sealed tissue. The LigaSure jaw was positioned approximately 1 cm above the ovary to ensure complete occlusion of the jaws and achieve optimal results. Frequent cycles of full cautery dissection were performed on the mesovarium, mesosalpinx, and appropriate ligaments. Each point of tissue was sealed twice at a distance equal to the jaw width of the device before being cut midway.

Following the complete dissection of the ovary, the LigaSure instrument was removed, and the tissue was observed for any signs of hemorrhage. Prior to releasing the ovary, the LigaSure instrument was used to seal the tissue but not to cut it. This allowed the ovary to remain suspended until it was accessed with a grasping instrument for removal from the abdomen. Once the ovariectomy procedure was completed, the ovary was extracted through the second port's skin incision by grasping it with Babcock grasping forceps. In cases where the ovaries were large, an additional extension of the incision was made to facilitate their removal. The right secondary port was always used to remove the ovaries.

Post ovariectomy, the abdominal incisions were routinely sutured in layers. The abdominal muscles were sutured as a single layer using number 0 Vicryl suture in a simple continuous pattern. The subcutaneous tissue was closed with Vicryl number 0 using a simple continuous pattern, and the skin was closed using number 1 polypropylene in simple interrupted stitches.

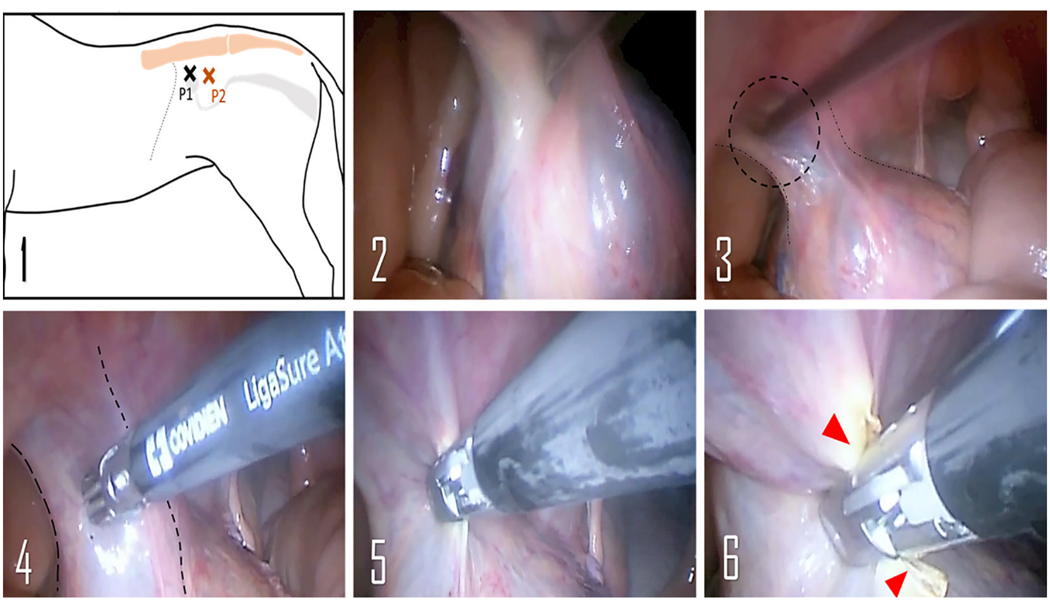

Figure 2: Two-port laparoscopic ovariectomy in a standing mare using LigaSure via a single surgical port. (1) The site for the laparoscope (P1) and LigaSure port (P2), (2) identification of the left ovary, (3) infiltration of the local anesthetic, (4) insertion of the LigaSure into the mesovarium, (5) initiation of the desiccation process, and (6) the cutting process.

2.3. Three-Port Laparoscopic Ovariectomy (Figure 3)

The technique was applied to six mares. A 10 mm main port for the laparoscope was positioned 3 cm caudal to the 18th rib at the level of the tuber coxae. Two access points for instruments were created in the paralumbar fossa. The first portal was situated at the upper border of the internal abdominal oblique muscle, halfway between the last rib and the tuber coxae. The second portal was located 8 cm below the first portal. Three 10 mm trocar-cannula units (AED, Germany) were inserted into the incisions of the main and secondary portals. Carbon dioxide (CO_2) was used to inflate the abdomen at a rate of 6 L/min until a pressure of 12 mm/Hg was achieved.

A 0-degree laparoscope was used to explore the abdomen. An anesthesia needle was inserted through the secondary cannula, and the ovarian pedicle was infiltrated with a 1% lidocaine solution (20 ml). Two 10 mm instruments were utilized: a Maryland grasping forceps (EndoMed Systems GmbH, Germany) held by the surgeon's left hand through the ventral portal and a bipolar cautery device (5×330 mm bipolar flat, U handle, BSC, India) operated by the surgeon's right hand through the dorsal port (the surgeon being right-handed). Additionally, 10 mm curved scissors (EndoMed Systems GmbH, Germany) were alternated with the bipolar electrocautery device. Following anesthesia, the ovarian pedicle was grasped for stabilization, and a bipolar device was used to cauterize the tissue cranially to caudally. The power of the cautery device was adjusted based on the color of the dissected tissue. A white to yellow color indicated a good seal, while a brown to blackish color indicated tissue burning. After each cycle of tissue cauterization, the bipolar device was replaced with scissors to perform dissection. Two to three cycles of cautery and dissection were performed until the ovary was completely freed. Each point where tissue dissection was required was cauterized twice at a distance equal to the jaw of the device, and scissors were used to cut at the midpoint. The ovaries were then removed through the incisions of the secondary ports. The incisions through which the ovaries were removed were enlarged to the needed length to remove the ovaries from the abdomen.

The abdominal wall was sutured in a single layer, encompassing all three muscle layers, using a Vicryl number of 0 in a simple continuous technique. The subcutaneous tissue was closed in a similar manner. The skin was closed with polypropylene number 1 in a simple interrupted pattern. Adhesive dressings were applied over the surgical sites and changed daily.

2.4. Postoperative Measures

The mares were provided with soft food two hours after the surgery, and they were discharged from the hospital 24 hours after the procedure. Postoperatively, systemic antibiotics (penicillin/streptomycin) (8 and 10 mg/kg) were administered intramuscularly, and phenylbutazone (4.4 mg/kg) was administered intravenously once daily for three consecutive days, except for animals that showed general illness or local surgical site infection. Veterinary care and stall rest were advised.

2.5. Evaluation Process

Local veterinarians were asked to monitor the animals during the complete evaluation process. Their precise observations were transferred to the operating surgeon via telephone calls. All the evaluation parameters, including the technical feasibility, operative time, degree of ease, postoperative pain, and cosmetic appearance of surgical wounds, were recorded.

2.6. Histopathological Examination

The excised ovaries were collected, grossly examined, and photographed to record the ovarian structures and/or any alterations. The ovaries were then cut longitudinally into two equal halves and fixed in 10% formalin solution immediately after collection for 48 hrs. The specimens were then processed by the paraffin embedding method, sectioned at 5-7 µm, and stained with hematoxylin and eosin according to Bancroft and Gamble [39].

2.7. Statistical Analysis

Statistical analysis was performed to determine the standard error of the means by using SPSS software (SPSS, Version 28.00, IBM, USA).

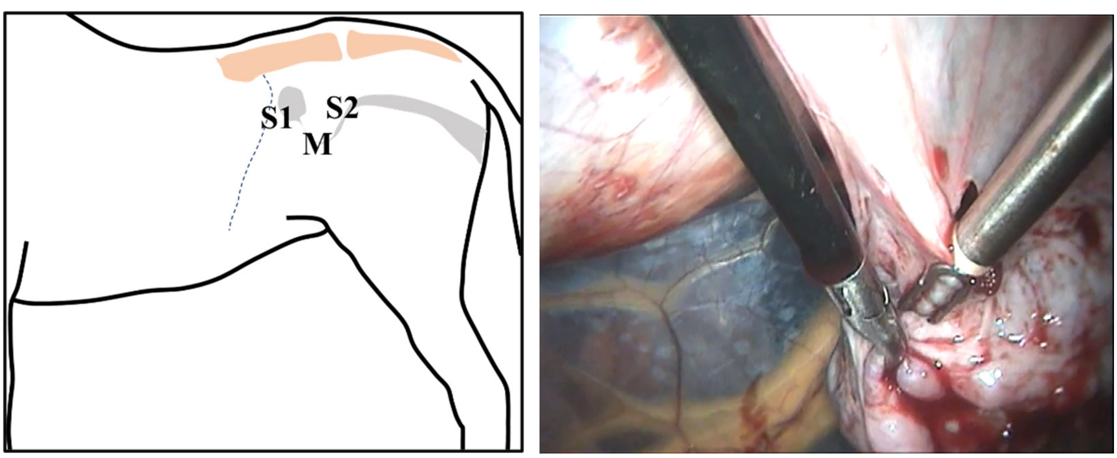

Figure 3: Three-port standing laparoscopic ovariectomy using bipolar electrodes and Maryland grasping forceps. (S1) is the grasping forceps port, (M) is the laparoscope port, and (S2) is the bipolar port.

3. Results

Unilateral laparoscopic ovariectomy was successfully performed on twelve mares using two different techniques: the two-port LigaSure technique (n = 6) and the three-port bipolar technique (n = 6). In terms of technical feasibility, both techniques were feasible, but the two-port technique was more feasible. In terms of safety, the use of LigaSure was safer than the use of bipolar lenses, with no intraoperative complications recorded. However, tissue coagulation until blenching, shrinkage, and burning were observed in all animals treated with bipolar materials, and tissue blood oozing occurred in 3 patients, which was managed by further cauterization. Bleeding was observed in 3 patients, but it was controlled by additional cauterization attempts. The use of bipolar cauterization resulted in smoking and temporary unclear visualization of the target tissue. No injuries to internal organs were recorded with either technique.

The mean operative time for the LigaSure group was 30.65 ± 2.85 minutes, while the bipolar cauterization technique took longer, with a mean surgical time of 43.1 ± 4.25 minutes. Both techniques were considered easy, with the operating surgeon rating all LigaSure procedures and 5 out of 6 bipolar procedures (90%) as easy (score = 1). One of the bipolar surgeries (16.6%) was rated as difficult (score = 2) due to poor tissue manipulation and required the longest surgical time (51.34 minutes).

Regarding postoperative pain, 2 out of 6 animals (33.3%) in the LigaSure group experienced moderate postoperative pain (score = 6) that required intravenous administration of flunixin meglumine for an additional 2 days. One mare (16.6%) experienced severe postoperative pain (score = 9) accompanied by fever and anorexia, which resolved by day 7 after surgery. In the bipolar group, 5 out of 6 animals (83.3%) experienced moderate postoperative pain (score = 6) 5 days after surgery. Two of the 6 animals (33.3%) experienced general illness, fever, or severe postoperative pain (score = 8), which resolved with systemic treatments by day 9 after surgery. An evident increase in pain score was observed with the three-port approach.

In terms of surgical wounds and cosmetic appearance, 2 out of 6 animals (33.3%) in the LigaSure group exhibited wound swelling and seroma, as well as local inflammation without infection. One animal out of 6 (16.6%) showed mild swelling and localized subcutaneous emphysema. Local abnormalities at the surgical site disappeared in all animals by day 14, and the sutures were removed. In the electrocautery group, wound infection was reported in two animals (33.3%) on day 3; this infection was treated topically and fully resolved by day 9 postoperatively. All animals exhibited a good cosmetic appearance three weeks after surgery. Additionally, all the mares were able to resume normal activity within 15-21 days after surgery, and the owners were satisfied with the cosmetic outcomes.

Overall, two-port laparoscopic ovariectomy using LigaSure proved to be a feasible, safe, and beneficial alternative to the three-port electrocautery technique. Grossly, the ovaries were 10-15 cm in diameter. On the cut surface, GCTs were polycystic, solid, or a combination of both. The cyst fluid was sanguinous or serous. The solid areas were white and grayish to yellow and orange, depending on the degree of hemorrhage that occurred within the tumor. According to a microscopic examination of the ovaries, 11 of the examined samples exhibited granulosa cell tumors. They were mostly polycystic and histologically consisted of cysts that resembled disorganized attempts at follicle formation, accompanied by a prominent supporting stroma of spindle cells interpreted as theca cells. Within the follicular structures, multiple layers of cells that resemble granulosa cells, often palisading at the periphery, were observed. The last ovary displayed a multifocal pattern with rosettes, where groups of eosinophilic materials formed Call-Exner bodies with radiating granulosa cells. These cells were arranged in rosettes that clustered together and were bound by stromal cells **(Figure 4)**.

4. Discussion

The present study aimed to evaluate the feasibility, safety, and benefits of a two-port laparoscopic ovariectomy using LigaSure compared to the three-port electrocautery technique in standing mares. The results of the study demonstrated that LigaSure was a feasible option for performing standing laparoscopic ovariectomy in mares, which is in line with previous studies **[11,40]**. LigaSure provided simultaneous coagulation and cutting of tissue, and its ease of use, along with the microprocessor that adjusts optimal heat and sealing time, made it more feasible than bipolar electrocautery, which requires manual adjustments for optimum tissue sealing **[41,42]**. The single-device approach with LigaSure simplified the procedure and reduced the operative time compared to the use of multiple tools, such as Babcock grasping forceps, bipolar electrodes, and scissors. Moreover, the use of a single operating port for LigaSure was sufficient to seal and cut the ovarian pedicle and broad ligament, eliminating the need for additional grasping tools, reducing invasiveness, and improving cosmetic outcomes. These findings support the convenience and benefits of the two-port approach in terms of feasibility, ease of use, shorter surgical time, minimal postoperative pain, and improved cosmetic appearance, as reported in previous studies **[43–47]**.

The LigaSure vessel-sealing device allowed for simultaneous grasping, coagulation, and cutting of tissue, enabling the surgeon to operate with a single port without the need for instrument exchange. LigaSure utilizes a combination of pressure and energy to effectively seal larger vessels up to 7 mm in diameter with minimal thermal spread, potentially reducing collateral tissue damage and offering faster dissection times **[41]**. In the current study, where the granulosa cell tumors were midsize, ≤15 cm in diameter, and highly vascularized, LigaSure was optimal for removing tumors with minimal intra- and postoperative complications. In contrast, while bipolar electrosurgery is versatile and capable of a wide range of surgical functions, such as cutting, coagulating, and dissecting tissues, it poses a slightly elevated risk of causing tissue charring and inadequate hemostasis. This heightened risk has contributed to intraoperative complications, emphasizing the limitations of bipolar electrocautery for mid-size ovariectomy. The results of the present study aligned with previous reports of using bipolar electrocautery to control bleeding limited to small blood vessels up to 3 mm **[48]**. The anatomical position of the equine ovaries facilitated accessibility without requiring extra grasping instruments, and the use of LigaSure reduced the technical demands and the need for mesovarium dissection, leading to a shorter learning curve and reduced operative time **[21,49,50]**.

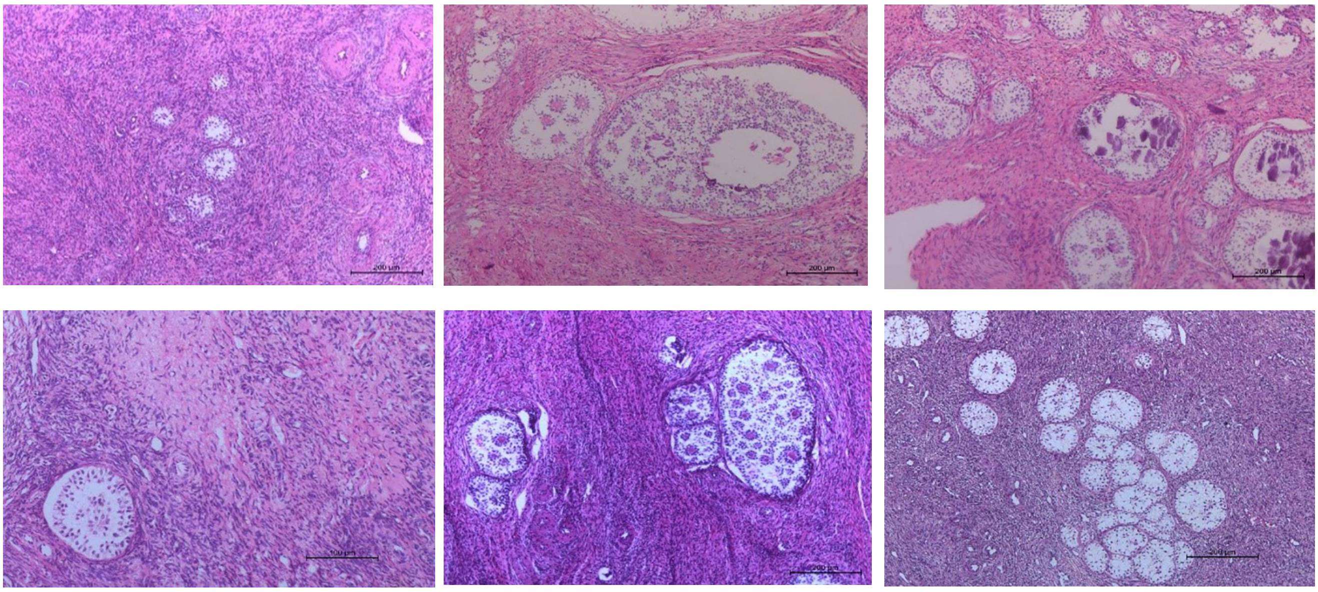

Figure 4: Photomicrographs of ovarian sections from studied mares (different ages) showing multifocal coalesced granulosa cell tumor bounded by fusiform stromal cells in a whorl-like arrangement (H&E; bar = 200 μm).

The study also revealed that the two-port laparoscopic ovariectomy technique using LigaSure resulted in a shorter mean operative time and decreased postoperative pain in the first 12 hours. The reduced number and sizes of the ports contributed to minimal scarring and improved cosmetic outcomes, as observed in other studies on reduced-port surgeries in animals and humans [51–54]. No major intraoperative or postoperative complications were encountered during the study, indicating that LigaSure provided a quick and secure method of vessel hemostasis, leading to successful standing laparoscopic removal of normal ovaries. The device demonstrated superior performance in terms of burst pressure, sealing time, thermal spread, and smoke production compared to bipolar cautery or ultrasonic devices [55–57]. Furthermore, the two-port laparoscopic ovariectomy technique was associated with reduced analgesia requirements and faster recovery time, leading to lower overall costs. The less invasive nature of the technique, with smaller incisions and improved cosmetics, was also advantageous [58–60].

5. Conclusions

In summary, the two-port laparoscopic ovariectomy technique using LigaSure has been proven to be a safe and efficient method for achieving hemostasis of the mesovarium. It has also been shown to be technically straightforward, leading to shorter operative times than the traditional three-port electrocoagulation technique, especially in cases involving medium-sized granulosa cell tumor ovaries.

Authors' Contributions

M.W.E. advised the project, and A.F. performed and interpreted the results of the histopathological examination. M.W.E. and A.N.E. performed the experiments, collected the data, and prepared the figures. M.W.E. and A.N.E. discussed the results and reviewed and edited the manuscript. M.W.E. and A.N.E. contributed equally to this work and shared the first authorship.

Data Availability

All the data collected or analyzed during this study are included in this published paper.

Funding

No funding was received to support this study.

Conflicts of Interest

The authors declare no competing interests.

Ethical Approval

The present study adhered to ethical protocols and received approval from the Faculty of Veterinary Medicine, Damanhour University (Ref. No. A02/2023). Surgical procedures were performed at the Animal Panorama Center of Excellence, Faculty of Veterinary Medicine, Damanhour University.

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