

Association of Primate Veterinarians' Guidelines for Laparoscopic Reproductive Manipulation of Female Nonhuman Primates in Biomedical Research

Purpose

The use of laparoscopic surgical techniques has become increasingly prevalent for reproductive manipulations in female nonhuman primates (NHP) in biomedical research. Laparoscopic approaches are often considered minor procedures thus reducing the number of major surgical procedures an animal will undergo during its lifetime. The Association of Primate Veterinarians (APV) supports the replacement of open surgical techniques with minimally invasive, laparoscopic approaches when possible to minimize pain and distress while also reducing post-operative recovery times. Replacing laparotomy with laparoscopy fulfills the refinement component of the 3Rs. The decision to pursue, approve, and utilize laparoscopic techniques is dependent on the institutional animal care and use committee (IACUC), the surgeon's skill with laparoscopy, available equipment, and establishment of a close working relationship between the veterinary and investigative staff. This document aims to provide NHP researchers, IACUC members, and veterinarians with guidelines for considering and conducting research involving laparoscopic reproductive manipulations in female NHP.

Background

Laparoscopy is a surgical procedure performed in the abdominal cavity through small incisions (usually 3-10 mm) with the aid of a camera, or laparoscope. Laparoscopy has been used in both human and NHP medicine for a variety of reproductive procedures. Common reproductive manipulations performed via laparoscopy in NHP include ovarian follicle aspiration for oocyte collection, ovariectomy, uterine biopsy, diagnostic laparoscopy, and embryo transfer. Laparoscopy is also utilized for more specialized reproductive manipulations, induction of disease models, and therapeutic interventions in NHP.

Given the smaller incisions in comparison to an open laparotomy, there is generally less tissue inflammation and decreased post-operative incision-related pain. Likewise, there is generally decreased hemorrhage and reduced post-operative risk of infection when compared to the same surgical procedures approached by laparotomy. Additionally, the risk of post-operative adhesion formation is decreased. Similar to human patients, these benefits combine to decrease the overall hospital stay, may decrease the need for extended post-operative analgesia, and, in NHP, reduce the time to return to social housing.

Appropriate training and proficiency in performing laparoscopic procedures is essential for achieving the benefits of these techniques over open techniques. Training opportunities for laparoscopic interventions are available through veterinary colleges and wet labs at the Academy of Surgical Research's

Annual Meeting. Primate veterinarians experienced in NHP laparoscopic techniques, board certified veterinary surgeons with experience in laparoscopy, and collaboration with human surgeons can also be useful sources for training when learning or developing laparoscopic techniques. Additional information about laparoscopic procedures is provided in the References section of this document.

IACUC Considerations

The 8th edition of the Guide states, "...major survival surgery ... penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection. Minor survival surgery does not expose a body cavity and causes little or no physical impairment...Laparoscopic surgeries...may be classified as major or minor surgery depending on their impact on the animal. For example, laparoscopic techniques with minimal associated trauma and sequelae...could be considered minor, otherwise...should be considered major. Although minor laparoscopic procedures are often performed on an "outpatient" basis, appropriate aseptic technique, instruments, anesthesia, and analgesia are necessary. Whether a laparoscopic procedure is deemed major or minor should be evaluated on a case-by-case basis by the veterinarian and IACUC."

For Animal Welfare Act covered species, including NHP, a major operative procedure under 9 CFR Section 1.1 is defined as "any surgical intervention that penetrates and exposes a body cavity or any procedure which produces permanent impairment of physical or physiological functions."

The IACUC, with input from the veterinary staff, is tasked with determining: 1) whether specific laparoscopic manipulations meet the definition of a major operative procedure or qualify as a minor procedure at their institution; 2) how frequently within a protocol and within an animal's lifetime should such procedures be conducted; and 3) the number of times a NHP may undergo such procedures. Depending on the complexity and consequences of the laparoscopic manipulation, single or multiple laparoscopic procedures may be approved within a protocol and within the lifetime of a research animal. These determinations should be made after careful consideration of the degree of manipulation associated with the procedure, and whether major physiologic impairment, or peri-operative or long-term consequences, such as pain and distress, are expected. As a general rule, in most cases, ovarian follicle aspiration with oocyte collection, diagnostic laparoscopy, and embryo transfer are minor surgeries due to the absence of negative impact on an animal's normal physiology. Ovariectomy, on the other hand, and any other procedures causing physiologic

impairment (e.g., loss of reproductive ability), are considered major surgery unless otherwise determined by the IACUC.

The 8th edition of the Guide also states that, “The institution should provide appropriate education and training to members of research teams . . . to ensure that they have the necessary knowledge and expertise for the specific animal procedures proposed and the species used.” The APV strongly encourages the IACUC to review the training and proficiency of the members of the research team that perform the laparoscopic procedures to ensure they uphold the standards described in the Guide.

Animal Selection

Female NHP selected for protocols involving laparoscopy are generally sexually mature with or without a history of active reproduction or reproductive manipulation. Routine screening of candidates for protocols involving laparoscopy should include a complete medical history review as well as a physical exam with a complete blood count and chemistry panel, if recent blood work has not been performed. History review should include an evaluation of previous births and reproductive manipulations with special attention paid to any complications or sequelae associated with these events. Abnormalities noted during previous reproductive procedures including aberrant anatomy, adhesions, or cysts may preclude assignment of an animal to a study that includes laparoscopy. A history of a normal menstrual cycle may also be important depending on study aims. Physical examination should include a reproductive evaluation with bimanual (rectal and abdominal) palpation of uterine structures, which normally move freely in the caudal abdomen without restriction. When selecting animals for studies involving laparoscopic reproductive manipulations, consideration of the animal’s future use in research and breeding may be warranted if physiological impairment (e.g., reproductive impairment) is expected.

Procedure Considerations

Laparoscopic technique, equipment, and pre-operative evaluation will vary depending on the research goals and procedures being performed. The procedure may be modified for certain species or for animals with previous manipulations. For species that classically develop abdominal adhesions, such as great apes, pre-operative ultrasound may be warranted to determine if a para-midline versus supraumbilical midline approach is preferable to avoid existing tissue attachments. Animals that have undergone previous reproductive manipulations or laparotomy may have midline adhesions that necessitate use of a para-midline laparoscope port to allow appropriate visualization (see References).

A variety of intra-operative complications have been linked to laparoscopic manipulation in humans and animals. Complications may be associated with entry into the abdomen, creation of the pneumoperitoneum, positioning, and/or visualization/manipulation. Port incisions that are too large may result in loss of insufflation and difficulty maintaining appropriate visualization of the reproductive tract. Incisions should be as small as possible to allow placement of the port without damage to the surrounding tissue to minimize CO₂ leakage. Placement of ports with the aid of trocars may result in mechanical trauma, including injury to major vessels or the gastrointestinal tract, abdominal wall hematoma, perforated bladder, hernia, and/or ureteral injury. These structures may also be damaged during manipu-

lation of the abdominal contents by laparoscopic instruments if care is not taken when handling tissues or if visualization is poor making identification of abdominal structures difficult. Pneumoperitoneum, created by insufflation with CO₂ gas to improve visualization and manipulation of abdominal contents, has also been associated with intraoperative complications such as respiratory acidosis, deep vein thrombosis, subcutaneous emphysema, gas embolism, reduced dynamic lung compliance, and increased peak inspiratory and plateau pressures. In humans, shoulder pain due to irritation of the diaphragm and/or stretching of the phrenic nerve has also been noted following laparoscopic procedures. Animals in a Trendelenburg position (supine position in a 15 to 30-degree incline with the feet elevated above the head; standard for female laparoscopic manipulation) paired with CO₂ insufflation of the abdomen may experience an increase in mechanical impedance to lung inflation, therefore mechanical ventilation is recommended.

Post-Surgical Considerations

Post-operative monitoring should be provided continuously post-procedure until the patient is able to maintain itself consistently in an upright position. In addition, post-operative monitoring, including evaluation of basic NHP physiologic and behavioral parameters, should be conducted daily for 3-7 days post-procedure. Post-operative analgesia for routine laparoscopic manipulations should be provided for a minimum of 48-72 hours following the surgical procedure and may be accomplished with opioids, NSAIDs, or a combination based on veterinary discretion.

Short-term complications associated with laparoscopy are generally associated with the surgical incisions; however, post-operative abdominal discomfort, particularly from increased abdominal pressure during insufflation, or hemorrhage may occur. Incisions should be monitored closely in the post-operative period for erythema, inflammation, and discharge, which may be indicative of an incision site infection. Fascial dehiscence and herniation of abdominal contents through the incision sites are also possible; although, due to the small size of the incisions, omentum is generally the only abdominal tissue present in laparoscopic incisional hernias. Edema at the incision site may be mistaken for herniation; however, edema will resolve spontaneously.

Long-term complications associated with laparoscopic procedures center on impaired fertility, endometriosis, and adhesion formation. Fertility may be compromised by trauma or damage to the ovaries, oviducts, uterus, or associated vasculature during the laparoscopic procedures. Adhesion formation is a common sequela to laparoscopic reproductive manipulation in NHP. Adhesions are generally minor involving small omental attachments to the ovaries, uterus, or abdominal port sites. More extensive adhesions are possible and may distort normal reproductive anatomy. The presence of adhesions may be evaluated during follow-up physical examinations through the use of bimanual palpation and reproductive ultrasound. Strict attention to hemostasis at the incision/trocar entry sites and thorough flushing of the abdomen following laparoscopic manipulation to remove blood and extraneous fluid may reduce the risk of adhesion formation. Flushing of the abdomen may also reduce the risk of endometriosis development in the case of transuterine laparoscopically-guided manipulations (e.g., uterine biopsy). Overall, when performed correctly in healthy patients, the risk of post-surgical complications is very low.

Record Keeping

Detailed records inclusive of the surgical procedure, any perioperative findings and/or complications, anesthetic and analgesic agents, doses, and administration routes should be completed. Records should also include comments capturing the animal's behavior and description of objective (e.g., body weight shifts, fluid volume and number of biscuits consumed, presence/absence of feces and urine) and subjective (e.g., level of activity, alertness, responsiveness, overall disposition) clinical parameters.

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