Laparoscopic artificial insemination with frozen-thawed semen in a bitch

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Summary

Laparoscopic artificial insemination (L-AI) in a bitch is described. Breeding management of a six-year-old Rottweiler bitch was accomplished by blood progesterone and luteinizing hormonal assays. For L-AI, the bitch was anesthetized, and the surgical site prepared under aseptic techniques. Two incisions about 1.5 cm in length were made, one for the laparoscope and the other for the instrument portal. The uterus was elevated to the ventral abdomen where a 20g indwelling catheter was threaded into the body of the uterus and fed into each horn individually delivering 2.5ml of frozen-thawed semen. The incisions were closed, and the bitch discharged two hours after L-AI. The bitch's pregnancy was confirmed by transabdominal ultrasonography. On day 60 after insemination the bitch vaginally delivered two stillborn puppies. Because of abdominal contractions without delivery of remaining fetuses, the bitch was taken to a veterinary clinic where a cesarean section was performed. Five live and three dead puppies were delivered. Laparoscopic artificial insemination provides another option for depositing frozen-thawed semen in the canine uterus. The main advantage of L-AI compared to traditional surgical AI is the quick recovery.

Keywords: Laparoscope, artificial insemination, frozen-thawed semen, bitch

Case presentation

A six year-old female Rottweiler was presented for pre-breeding examination and artificial insemination using frozen thawed semen. Three years earlier the bitch was inseminated by transcervical insemination (TCI) but pregnancy did not occur. Two years earlier the bitch was bred by natural service resulting in four puppies delivered via cesarean section These breedings were with different sires. The bitch was current on vaccination and was negative for *Brucella canis*. The bitch was not on any medication and did not have any previous medical conditions.

On physical examination, the bitch was bright, alert and responsive. She was in desirable body condition (47 kg; 3.5/5 BCS), her hair coat was soft and shiny, no musculoskeletal conformation abnormalities were noticed. Ears, eyes, nose and mouth were clean, clear and free of any discharge or debris. No lymph nodes were palpably abnormal. Her rectal temperature, respiratory rate and heart rate were within normal limits. Her mucous membranes were pink and moist with a capillary refill time of <2 seconds. Mammary chains were normal. A mild clear, viscous discharge from vulva was noted. The vulva was swollen and felt warm to the touch. Vaginal examination revealed a moderate stricture at the vestibule vaginal junction approximately 3 cm. from the vulvar opening. The stricture was not considered to be sufficient to prevent normal whelping. No pain was elicited with flexion and extension of all joints. Abdominal palpation was within normal limits. Neurologic examination of the cranial nerves and proprioception were within normal limits.

Breeding management

Blood samples were collected for progesterone (P4) and luteinizing hormone (LH) assays. Progesterone was assayed by radioimmunoassay, whereas the LH was detected by enzymeimmunoassay(Witness LH test kits, Synbiotics, Kansas City, MO). Cotton-tipped swabs were used to collect vaginal cells which were smeared on to microscope slides, stained with a commercial Romanowsky stain variant (Diff Quik, Jorgensen Labs, Loveland, CO) and evaluated microscopically.

Transcervical insemination was attempted using an endoscope (Karl Storz, Tuttingen, Germany). Many vaginal folds were visualized, further characterizing estrus activity. Several attempts were made over a 15 minute period to pass a catheter through the cervix into the uterus but were unsuccessful.

Laparoscopic artificial insemination

The bitch was premedicated with hydromorphone (0.1 mg/kg) and dexmedetomidine (2 mcg/kg) as a mixed intramuscular injection. Anesthesia was induced with intravenous injections of midazolam (0.2 mg/kg), ketamine (3.6 mg/kg), and dexmedetomidine (1 mcg/kg), and maintained by isoflurane. The surgical site, including the ventral abdomen from the xyphoid to the pubis was prepared using routine aseptic techniques and draped. Two ventral midline incisions were made, one for the laparoscope and one for an instrument portal, both approximately 1.5 cm in length. The visual portal was located immediately caudal to the umbilicus and the caudal instrument portal 3 cm cranial to the pubic rim. The uterus was grasped with an atraumatic tissue forceps, elevated to the ventral abdominal wall where a 5F central silicon catheter (V-Cath Picc, Neomedical, Mila International Inc, Erlanger, KY) was placed transabdominally, utilizing the 18 g over-the-needle peel-away introducer of the catheter kit. The introducer entered the body of the uterus in an oblique angle, aiding cranial advancement of the catheter. The catheter was advanced 10-15 centimeters, and 2.5ml of properly thawed and prepared semen delivered into each horn. The abdomen and its organs were free of any adhesions or obvious lesions. The incisions were closed in three layers including the abdominal wall, subcutis and intradermal. The bitch recovered uneventfully, and was free of any outward signs of pain including posturing, vocalizing and general attitude. She was discharged two hours postoperatively. No further pain medication was deemed necessary.

Outcome

On day 60 after insemination the bitch vaginally delivered two stillborn puppies. Because of abdominal contractions without delivery of remaining fetuses, the bitch was taken to a veterinary clinic where a cesarean section was performed. Five live and three dead puppies were delivered.

Discussion

To our knowledge, this is the first report on successful outcome of a laparoscopic AI (L-AI) with frozen-thawed semen in the bitch. Laparoscopic AI with fresh canine semen was reported by Silva, et al.[1] In dogs, laparoscopic surgery has been associated with less pain and faster recovery, compared to open procedures.[2-4] In addition, intraoperative oxidative stress,[5] C-reactive protein levels,[6] and hemodynamic changes[7] are minimized with laparoscopy. Therefore, the minimally invasive nature of the surgery in this case may have contributed to the successful outcome. However, laparoscopic surgery entails limitations including the requirement for specialized equipment and skills. The unsuccessful attempts to catheterize the cervix in our case may have been due to nonpatency of the cervix as a result of a previous cesarian section.

In addition to challenging canine breeding management, AI requires additional efforts, especially AI with frozen-thawed semen. Frozen-thawed sperm is viable for a short time, therefore intrauterine insemination is recommended. Pregnancy rates of 10-60% are reported with vaginal insemination with frozen-thawed semen.[8-11] Semen deposition into the uterus via the vagina is difficult due to the unique cervical anatomy which is unfavorable for catheterization without visualization of the cervical os.[1,8,9,12,13] The long canine vagina, which can be up to 29cm long in large breeds of dogs[13] is another difficulty in reaching the cervix through the vagina. Due to these reasons, endoscopic catheterization or TCI is commonly used for canine artificial insemination with frozen-thawed semen. Numerous TCI techniques are reported.[15-18] However, TCI requires practice and sometimes can be challenging.[14,19] Surgical insemination in the uterus is another technique to deposit semen directly into the uterus.[20] The main disadvantage of surgical insemination is the time required for surgical preparation and post-surgical recovery of the patient. Laparoscopic artificial insemination offers quick recovery. In the case reported, the bitch was released from the hospital two hours after L-AI.

Learning points

- Laparoscopic artificial insemination provides one more technique for depositing frozen-thawed semen into the canine uterus.

- The main advantage of L-AI compared to traditional surgical AI is rapid recovery.

Acknowledgements

The authors wish to thank Alpine Animal Clinic, Helena, MT for referring the case to the Veterinary Teaching Hospital at Washington State University, and to Dakota Woodward for attending the case.

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