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Comparison of Transcervical Insemination Versus Surgical Insemination Methods in Dogs at a Veterinary Clinic in Lincoln, Nebraska

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COMPARISON OF TRANSCERVICAL INSEMINATION VERSUS SURGICAL INSEMINATION METHODS IN DOGS AT A VETERINARY CLINIC IN LINCOLN, NEBRASKA

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Abstract

Artificial insemination paved the way for many advancements in reproductive technology. Two common artificial insemination methods recommended for use with frozen semen are transcervical insemination and surgical insemination. To better understand the conception rates of dogs for each method, data was collected from Hillcrest Animal Clinic. The data suggested that neither transcervical insemination nor surgical insemination was significantly better than the other. 50% of the dogs conceived when inseminated using the transcervical method, and 54.5% of the dogs conceived when inseminated using the surgical method. Based on the results, more data needs to be collected on both insemination procedures to gain a better understanding of conception rates for each. Additionally, studies need to be performed comparing conception rates of dogs with a combination of insemination methods or multiple insemination procedures.

Key words: artificial insemination, dog, transcervical insemination, surgical insemination, frozen semen, ovulation, conception rate

Literature Review

Artificial Insemination

Through the implementation of artificial insemination (AI) methods, reproduction and genetics have made significant advancements for many species including dogs. Since the introduction of artificial insemination, other technologies have been developed such as cryopreservation, sexed semen, regulation of the estrous cycle, collecting, freezing, culturing, and transferring of embryos, and cloning (*The history of artificial insemination: Selected notes and notables*). The history of artificial insemination begins with Lazzaro Spallanzani. Lazzaro Spallanzani performed the first artificial insemination in a dog in 1784, which led to the birth of three puppies (Ombelet & Van Robays, 2015). In 1897, artificial insemination in rabbits, dogs and horses was recorded by Heape, a reproductive biologist. Lastly, Ilya Ivanovich Ivanoff developed the methods used in human medicine today from his studies on artificial insemination conducted by using rabbits, dogs, poultry and other farm animals.

Frozen Semen AI

Artificial insemination with frozen semen in canines resulting in pregnancy was first documented in 1957, 1970, 1972, and 1973 (Johnston et al., 2001). However, in 1989, less than 500 canine pregnancies from frozen semen insemination were documented due to low success rates resulting from the inability to determine the day of ovulation, the limited post-thaw life span of canine frozen semen, and the position of the cervix within the bitch. However, the advantages of utilizing canine frozen semen, such as capturing genetic material until the time of breeding and preserving genetic material after the donor has passed was enough to sustain the practice despite the high cost. Additionally, frozen semen can be collected from male dogs and stored long term,

allowing the semen to be shipped in canisters designed to hold liquid nitrogen or liquid nitrogen vapor in “dry shippers” to veterinary clinics across the United States. It is critical to assess the liquid nitrogen level in the tank to prevent the thawing of the semen straws once it has arrived to its destination. All shipped frozen semen should come with thawing instructions, the number of spermatozoa in an individual straw, and the percentage of the post-thaw progressive motility (Johnston et al., 2001). When using frozen thawed semen for artificial insemination, conception rates in bitches are highly volatile due to ovulation day, semen quality, buffer/extender, freezing method, insemination site, how many sperm, how many breedings, and normalness of the bitch’s reproductive tract (Johnston et al., 2001).

Determining Ovulation

Progesterone assay is used to determine the ovulation date in dogs (Kustritz, 2010). Prior to ovulation, serum progesterone concentrations gradually increase until maximum secretions of luteinizing hormone (LH) are achieved, resulting in a drastic jump from baseline values. Within 24 to 48 hours before ovulation, the average range of serum progesterone concentrations is about 2.0 to 2.9 ng/ml; whereas, on the day of ovulation, serum progesterone concentrations range from 4.0 to 10 ng/ml (Kustritz, 2010). Dogs are inseminated with frozen semen three to four days after ovulation (Stich, 2022). The wait time is necessary because a primary oocyte is ovulated in the canine species and 48 hours is required for the last division of a primary to a secondary oocyte.

Serum Progesterone Concentration (ng/ml)	Interpretation
Less than 1.0 1.0 to 2.0	Anestrus or early proestrus- recheck. LH peak has not yet occurred- recheck.
2.0 to 4.0	LH peak occurring, near ovulation- recheck.
4.0 to 10.0	Ovulation occurring.
Greater than 10.0	Ovulation has occurred.

Thawing Frozen Semen

Proper thawing techniques are critical for the success of artificial insemination. After semen is frozen and thawed, it loses viability through physical destruction of cells, osmotic change, dehydration, ice crystal formation, and processes during freezing and thawing that act like physiological changes that sperm endure to gain the ability to enter and fertilize an egg (Kustritz, 2010). Conception rates of frozen-thawed semen for vaginal, transcervical, and surgical insemination are as follows: 45%, 70%, 95%. The three essential components to a successful pregnancy are normal semen quality, timing of insemination, and the site of semen deposition (Johnston et al., 2001). A consensus was reached that higher conception rates and increased litter sizes were due to insemination within the uterus using thawed frozen sperm when compared to vaginal insemination using thawed frozen sperm.

Frozen semen contains ejaculated spermatozoa and extender, which is a fluid medium consisting of nutrients, buffers, anti-microbials, and cryoprotectants such as egg yolk and glycerol. Semen that is less than very good or even less than excellent quality should not be frozen. Semen quality is based on concentration, motility, and morphology. When inseminating, two to three straws of frozen semen are used (about 1.0 - 1.5 ml). The straws are placed in a warm water bath (98 degrees Fahrenheit) for 60 seconds. The straws are then cut, and the semen is emptied into a sterile tube. Finally, the semen is drawn up into a syringe in preparation for insemination.

Post-thaw motility is acceptable for use from 40% to 80% (*CHF and Zoetis reproduction series: Cryopreservation of canine semen*). A lower percentage (i.e. 40%) just means that a higher volume will be used, whereas if the percentage is higher (i.e. 80%), less volume is needed for insemination. Post-thaw motility is determined by placing a drop (about 20 microliters) of semen on a pre-warmed slide and cover it with a coverslip (Payan-Carreira et al., 2011). The sperm is evaluated by the average percentage of progressively motile spermatozoa in multiple fields of the slide. The total number of spermatozoa is dependent on multiple variables: breed size, age, testicular weight, and sexual activity (Payan-Carreira et al., 2011).

Once the sperm is thawed and prepared, insemination may occur. There are three types of insemination procedures used in dogs: vaginal, transcervical, and surgical insemination.

What is Transcervical Insemination?

Transcervical insemination (TCI) is a procedure that directly puts the semen in the uterus. It is performed with the dog standing and without anesthesia. The process consists of inserting an endoscope through the vagina via a vaginal catheter, moving it past the caudal end of the post-cervical dorsal median fold, identifying the cervix, and passing a urinary catheter into the lumen

of the cervix (Kustritz, 2010). Semen is then pushed through the urinary catheter via a syringe. Air insufflation is utilized to improve vision and/or assist in navigating the reproductive tract. The endoscope itself assists in straightening the cervical canal (Johnston et al., 2001).

TCI is less invasive than surgical (SX) and is mainly implemented when using frozen semen, fresh chilled semen, or poor-quality fresh semen (Kustritz, 2010). Frozen semen is only viable for a short period of time after thawing; therefore, it should be placed directly into the uterus. Additionally, three to four days post-ovulation frozen semen is inserted into the uterine tube to ensure secondary oocytes are present (Johnston et al., 2001). During a single heat cycle, TCI can be utilized multiple times, as well as in tandem with vaginal or surgical insemination.

What is Surgical Insemination?

Surgical insemination (SX) is a procedure consisting of introducing a 22-gauge needle or catheter into the lumen of the uterine body via a five cm midline incision on the abdomen and exteriorizing the uterus using a Snook ovariohysterectomy hook (Johnston et al., 2001). In order to limit the time that the thawed semen is at room temperature, semen should be thawed only when relatively ready to inseminate the bitch (Kustritz, 2010). After the semen has been drawn up into a three to six ml syringe, it can be pushed through the catheter, flowing into the uterine lumen distending both the uterine body and horns. Only about 0.25 ml is inserted initially to determine that the semen is in fact flowing into the uterine lumen (Johnston et al., 2001). Then, the entirety of the contents in the syringe can be expelled. The uterus is then returned to the body after insemination, and the abdomen is sutured close.

The objective of this study was to determine if differences existed in dog conception rates between transcervical insemination and surgical insemination procedures.

Materials and Methods

Hillcrest Animal Clinic, located outside Lincoln, NE, conducts a large number of canine artificial insemination procedures. The clinic uses both TCI and SX techniques. According to the American Kennel Club, TCI is recommended when using frozen or poor-quality semen. It is also recommended for use in larger breeds of dogs and in the case of a single breeding. SX is also recommended when using frozen or poor-quality semen; as well as, when dealing with females that are older, have a record of poor fertility, and uterine pathology (Rakosky, 2018). Determining which procedure to perform really comes down to either the request of the client or the post-thaw motility percentage. If post-thaw motility is a lower percentage (i.e. 40%), it is recommended to place the semen as close to the site of fertilization as possible, allowing the sperm to have better chance to reach the oocyte or an easier time reaching the oocyte despite the lack of motile sperm. Performing two TCIs or TCI and SX allows for another chance at fertilization. Multiple procedures increase the probability of pregnancy because there are multiple attempts at insemination after ovulation is confirmed.

At Hillcrest Animal Clinic, the bitch is first restrained in a standing position during the TCI procedure. Then, the bitch's vulva is cleaned using a few passes of iodine scrub on gauze squares followed by a few more passes with gauze squares soaked in warm, tap water. The TCI procedure noted above is then performed.

For SX, 20 minutes prior to induction, an injection of Acepromazine, which is a sedative and pre-anesthetic (Gollakner), is given along with an injection of Atropine to decrease drooling and secretions of the respiratory tract (Ruben, 2015). Next, a catheter is first placed in the bitch's leg through which Propofol is administered as a method of sedation and an induction of anesthesia (Glowaski & Wetmore, 1999). All injection and medicine dosages are calculated based on the

weight of the bitch. Then, the bitch is lifted from the floor onto the prep table, laying with the abdomen facing the ceiling. While on the prep table, the bitch is first intubated and hooked up to anesthesia. Next, the abdomen is clipped, removing hair from the incision site. The abdomen is also scrubbed with first with chlorhexidine and then alcohol continuing in an alternating pattern until the incision site is deemed clean. The scrubbing process is done by starting in the middle of the abdomen where the incision will be made and working outwards. While the abdomen is being scrubbed, the bitch's heart rate and respiratory rate are being monitored. The bitch is then disconnected from the anesthesia and swiftly moved to the surgery room table. After hooking the bitch back up to anesthesia, fluids are connected to the bitch's catheter. A heart monitor is also attached to the bitch's tongue prior to surgery. The veterinarian begins by gloving up and placing a surgical drape over the incision site. After a hole has been cut in the surgical drape, the exact protocol described in this paper is followed by the veterinarians at Hillcrest Animal Clinic. The actual procedure aspect of the SX lasts about 30 to 45 minutes.

Data on completed artificial insemination procedures was collected by running a usage report through Hillcrest Animal Clinics' software. Separate reports were generated to identify dogs that had undergone either a TCI or SX. The lists of dogs had to be filtered through to isolate only the dogs bred with frozen semen. Then, an Excel sheet was formulated containing information such as owner contact information, the dog's name, animal identification, breed, age, weight, method of insemination, post-thaw motility, the volume of the semen used, whether the dog became pregnant, and how many total puppies were born. Each owner was contacted to collect data pertaining to whether their dog had become pregnant and how many puppies their dog had. All other information was collected through the animal records at Hillcrest Animal Clinic. Statistical

comparisons were conducted on this data set using Welch's t-tests, as there were unequal numbers of "pregnant" and "not pregnant" samples.

Results

This experiment focused on medical procedures where the dog had a single TCI or a single SX. However, Hillcrest Animal Clinic often recommends two other options to their clients in addition to individual insemination methods: two TCIs or one TCI and one SX. This resulted in limited data being available for single TCI procedures compared to SX. Data was analyzed for 4 dogs that had TCI completed and 22 dogs that had SX completed. This lack of TCI information resulted in an inability to make the comparisons as planned.

When looking at the data collected from Hillcrest Animal Clinic, it was found that 50% of the bitches conceived when inseminated using the TCI method, and 54.5% of the bitches conceived when inseminated using the SX method (Table 1). These values are similar regardless of method. A study utilizing TCI and frozen semen in beagles resulted in a 87.5% conception rate and 6.3 mean number of fetuses (Hayashi et al., 2012). Abilene Animal Hospital also references a study conducted using TCI and frozen semen that resulted in a 83.3% conception rate and 7.5 mean number of fetuses (*Transcervical Insemination FAQ*). According to the American Kennel Club, if the estrus cycle has been managed properly, a 100% conception rate is achievable when using the SX method (Rakosky, 2018). Pleasant Valley Veterinary Services notes that if the bitch has little to no uterine pathology and the semen is of decent quality, a conception rate of 85% to 90% is achievable using the SX method (*Veterinarian in Washington, NJ US :: Surgical insemination*). Additionally, an average litter size of 6.5 were born when the SX method was used, but data was not available or was missing to determine the average number of puppies born

to dogs who had TCI completed. A study compared average litter sizes for 4 categories of dogs: Miniature (Yorkshire Terrier), Small (Shetland Sheepdog, Welsh Corgi Pembroke), Medium (Polish Mountain Hound, Bouvier des Flandres), and Large (Great Dane, Newfoundland). Average litter sizes ranged from 3.7 (Yorkshire Terrier) to 8.7 (Great Dane) (Fiszdon & Kowalczyk, 2009). The minimum litter size was 2 puppies (Yorkshire Terrier, Shetland Sheepdog, Welsh Corgi Pembroke), and the maximum litter size was 14 puppies (Great Dane, Newfoundland). In conclusion, litter size variability in different breeds of dogs can be linked to their body weight. Not enough data was collected to determine differences between the two methods: transcervical insemination and surgical insemination.

Table 1. Pregnancy rates (%) and number of puppies born to dogs inseminated using either transcervical (TCI) or surgical (SX) insemination methods

Item	**TCI[1]**	**SX[2]**
Pregnancy Rate, %	50	54.5
Number of Puppies	-	6.5

[1] Data represents 4 dogs

[2] Data represents 22 dogs

Due to the lack of differences between insemination methods, the dogs who conceived and those who did not were compared to determine if any difference could be noted in the success of insemination overall.

The average volume of the semen inserted into the bitches was very similar (p=0.62) for both those who conceived (1.1 ml) and those who did not (1.0125 ml; Figure 1). The volume of semen needed for insemination depends on the morphology, motility, and concentration of the sperm per mL (*Canine reproduction - faqs*). Ultimately, your veterinarian will determine how many straws of semen are needed for each breeding. As noted in figure 2, the average post-thaw motility of the semen inserted into the bitches was also very similar (p=0.77) for both those who conceived (68%) and those who did not (69%). An average post-thaw motility ranges from 40-60%, which indicates that of the original sperm cells, 40-60% have survived through the process of freezing and thawing (*Canine frozen semen FAQ* 2019). Additionally, post-thaw motility is acceptable for use from 40% to 80% (*CHF and Zoetis reproduction series: Cryopreservation of canine semen*).

Figure 1. Semen Volume of Pregnant Versus Non-Pregnant Dogs After Either a TCI or SX (n=26)

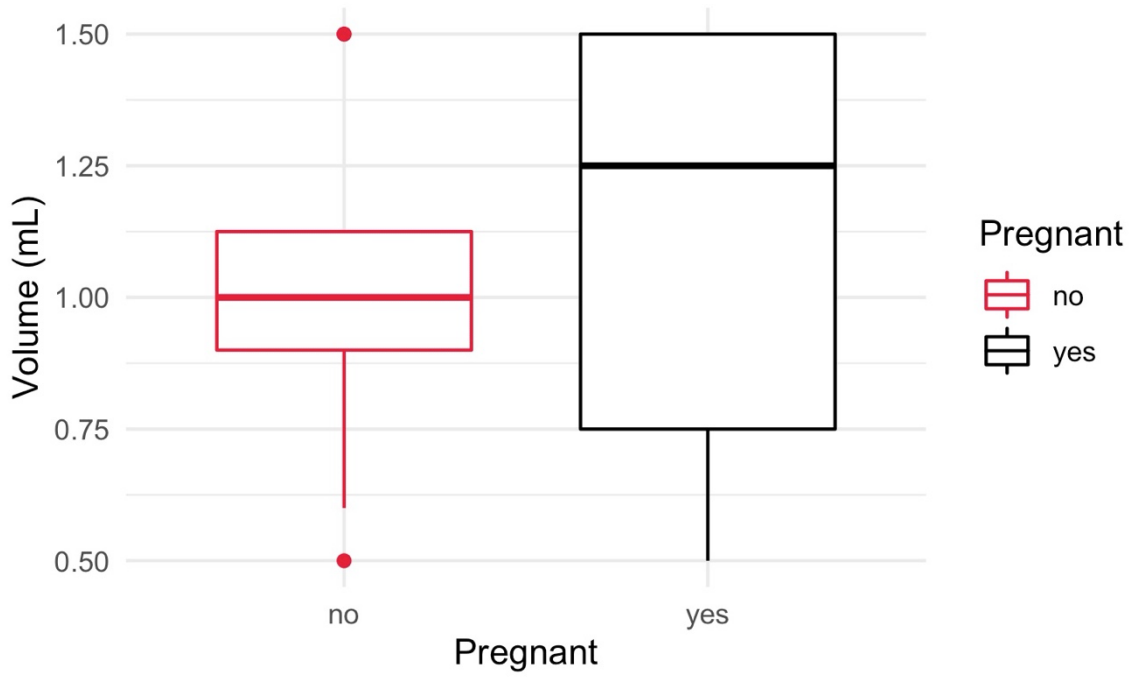
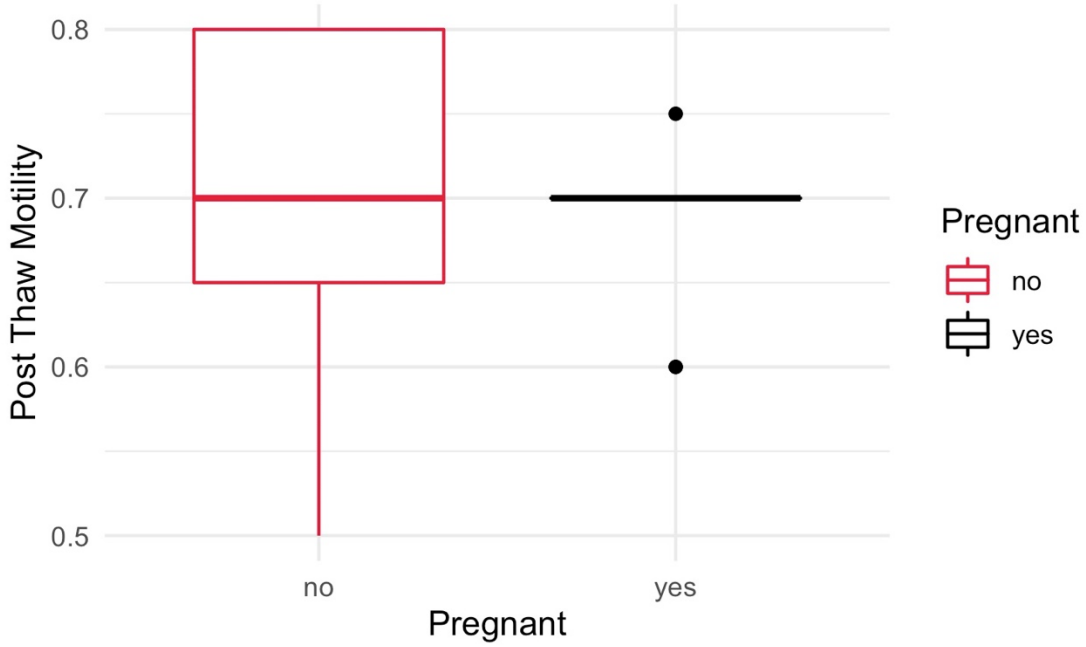
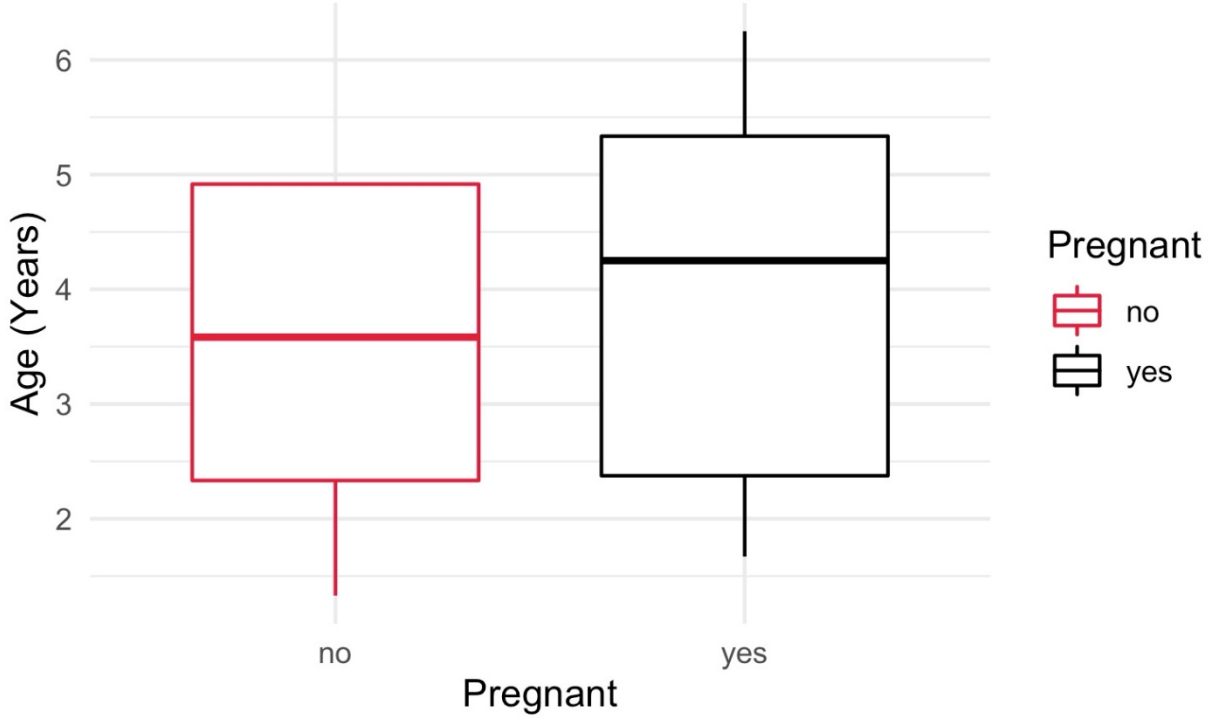


Figure 2. Post-thaw Semen Motility Rates of Pregnant Versus Non-Pregnant Dogs After Either a TCI or SX (n=26)



The average age of the bitches was relatively close (p=0.60) for both those who conceived (3.94 years old) and those who did not (3.47 years old; Figure 3). However, there was a wider range of ages in those that conceives than those that did not. This may indicate that the age of the dog is less of a factor in conception rates. Data was not available for those that had a successful breeding previously or not.

Figure 3. Age of Pregnant Versus Non-Pregnant Dogs After Either a TCI or SX (n=26)



There are a variety of factors that may affect a bitch's ability to conceive beyond the insemination method used. Most of the time failure to conceive is due to poor timing. Other possibilities include physical abnormalities such as an infection or inflammation of the uterus, hereditary reproductive tract abnormalities, and masses like benign uterine polyps or malignant tumors (Barnette). The bitch should be tested for a brucella canis infection prior to breeding and deemed in good physical health by a veterinarian (Rakosky, 2019). Ensuring the bitch is kept at a healthy weight, is up to date on vaccinations, and is given heartworm and flea and tick preventatives safe for pregnant dogs is ideal to increase the bitch's ability to conceive.

Conclusion

Both insemination methods (TCI and SX) were similarly effective. At Hillcrest Animal Clinic, it is more common to use a combination of methods or multiple procedures to ensure success such as, two TCIs, or one TCI and one SX. While the data for a combination of methods was not analyzed in this study, the use of both methods may result in a higher conception rate.

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