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Best Practices and Strategies for Artificial Insemination in Captive White-Tailed Deer[1]

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For captive and farmed cervids across the U.S., artificial insemination (AI) is among the most common reproductive practices. This technique enables greater genetic exchange and diversity without the need to transport animals between farms or states. Currently, there are no standard protocols for AI in cervids, and most research relies on advances from other small ruminants, such as sheep and goats.

This publication provides best practices for artificial insemination (AI) in captive white-tailed deer and aims to inform managers and farmers on what to consider when choosing a reproductive strategy.

Timeline of Reproduction in Florida White-Tailed Deer

For captive cervids in Florida, the deer breeding season, or rut, generally begins in mid-fall and ends with conception between May and July. The timing of rut depends on many factors but is primarily driven by shortening day length after the summer solstice. The reduction of daylight induces hormonal changes in the bucks. However, other factors such as doe behavioral and hormonal changes as well as geographic location also influence the rut period.

The first phase of rut begins with bucks losing the velvet from their antlers. This period is primarily characterized by male-male sparring to establish dominance. The courting period begins approximately 4 to 6 weeks after the start of sparring. Male-to-male aggression is most common in this phase. In wild cervids, aggression is normally shown by locking antlers and pushing until one male submits to the dominant male. When two bucks are evenly matched, it is common for males to sustain injuries, including punctures to the face and eyes as well as broken necks or even death in extreme cases. In captive cervids, it is a common practice to remove the antlers of males before courtship begins to ensure the safety of bucks who are kept together in pens. During this time, does will also undergo behavioral changes, such as avoidance behaviors or initiating chase tests of bucks, and hormonal changes, including pheromone production. More information about rut can be found in the Additional Information section of this publication.

Semen Collection and Storage

Semen should be collected at the peak of rut when antlers fully harden and before they drop, or immediately before insemination. There are two methods of semen collection: using an artificial vagina or electroejaculation. When collected, semen should be off-white in color and must be processed immediately after ejaculation to ensure quality and sperm motility. Red or pink tinges suggest blood contamination from inflammation or injury, a yellow and dilute sample indicates urine contamination, and brown indicates a possible reproductive tract infection. If contamination is thought to have occurred, it is recommended to test the quality of the semen before insemination or processing for freezing because contamination can lead to lower fertilization rates, health issues in the doe, or complications in pregnancy. Contact a commercial laboratory or a local veterinarian to ensure semen samples are of high quality.

After collection, fresh semen samples can be used to inseminate does, or samples can be diluted and frozen for later use. When storing or diluting semen, it is important to consider the concentration of sperm. While there is not a standardized concentration for the preservation of cervid semen, the minimum for other livestock may be used to infer proper concentrations for white-tailed deer. In goats, the minimum sperm concentration for preservation is around 200 million motile sperm per straw (0.25 mL), while in cattle it is around 500 million motile sperm per straw. Although fertilization is possible with a low concentration of sperm, it is not recommended to dilute semen samples below 100 million motile sperm per straw because sperm degradation will occur when freezing and thawing. To dilute semen samples, several commercially available dilutants can be used, such as AndroMed®, BioXCell®, or Triladyl®. In field settings, skim milk or egg yolk can also be used as a dilutant for immediate transvaginal or transcervical insemination; however, this is not recommended for long-term storage or when performing laparoscopic insemination. Two recommended

procedures for freezing and diluting fresh semen samples are described below.

Ultra-Rapid Freezing Protocol:

- Dilute semen sample using a commercially available semen cryopreservation solution.
- Aliquot dilute semen into 0.25 mL or 0.5 mL insemination straws.
- Cool dilute samples in the refrigerator (approximately 4°C or 32°F) for 30 minutes.
- Submerge samples in liquid nitrogen or place directly on top of dry ice for 15 minutes.
- Recommended to store in liquid nitrogen for long-term storage or in an ultra-low temperature freezer (-80°C).

Slow Conventional Cooling:

- Dilute semen sample using a commercially available cryopreservation solution.
- Aliquot dilute semen into 0.25 mL or 0.5 mL insemination straws.
- Place samples into a refrigerator (approximately 4°C or 32°F) and cool for at least 2 hours.
- Place the straws fully covered in liquid nitrogen vapor for 10 minutes.
- Submerge semen straws in liquid nitrogen for long-term storage or place into an ultra-low temperature freezer (-80°C).

When preparing to inseminate with frozen sperm, do not heat shock the sperm or expose the semen to UV light. Once removed from a cold source, the semen straw should be placed in a 95°F (35°C) water bath for at least 45 seconds to thaw. However, it should not be left in the water bath for more than 10 minutes, because longer exposure to heat could cause sperm to degrade. After thawing, the semen straws should be dried with a paper towel and protected from temperature shocks and UV light. Insemination in does is typically conducted using an artificial insemination gun. The AI gun should be warmed before inserting the semen straw. To do this, rub the gun vigorously with a paper towel and hold it close to your body. When inserting the straw, pull back the plunger of the gun, insert the straw by holding the end with the cotton plug, cut the sealed side of the straw off with a straight cut using disinfected scissors, and slide the plastic sheath over the AI gun to lock it in place. Prior to insemination, make sure to keep the AI gun sheath clean by covering it with a sanitized covering. This prevents bacterial introduction to the cervix or uterus and reduces the potential for health complications. Specific information on handling AI equipment such as liquid nitrogen and AI gun kits can be found in the Additional Information section at the end of this publication.

Syncing Estrous Cycles in Does

The rut primarily indicates when bucks can breed; however, does will not breed until they are in estrus, also known as "heat." The estrous cycle is the period of hormonal changes that prepare the female for ovulation and breeding. Deer are considered monestrous animals, meaning they only come into heat once a year. When performing AI on multiple animals, it is important to synchronize the females' estrous cycles to ensure all females are ovulating at the time of insemination. This is achieved with a controlled intravaginal drug-releasing device (CIDR) containing the hormone progestin, a synthetic version of the naturally produced hormone progesterone. The CIDR is placed inside the vaginal vault, near the entrance of the cervix, and releases progestin for 14 days to aid in the formation of the corpus luteum in the ovary (Figure 1). Application of this device results in synchronization of estrus. At day 14, the CIDR is removed and 1 mL of a follicle-stimulating hormone is injected intramuscularly. This hormone is commonly called pregnant mare serum gonadotropin (PMSG) and is obtained through a veterinarian. PMSG induces ovulation where an egg (ovum) is released from the follicle of the ovary and travels to the uterine horn via the oviduct (Figure 1).

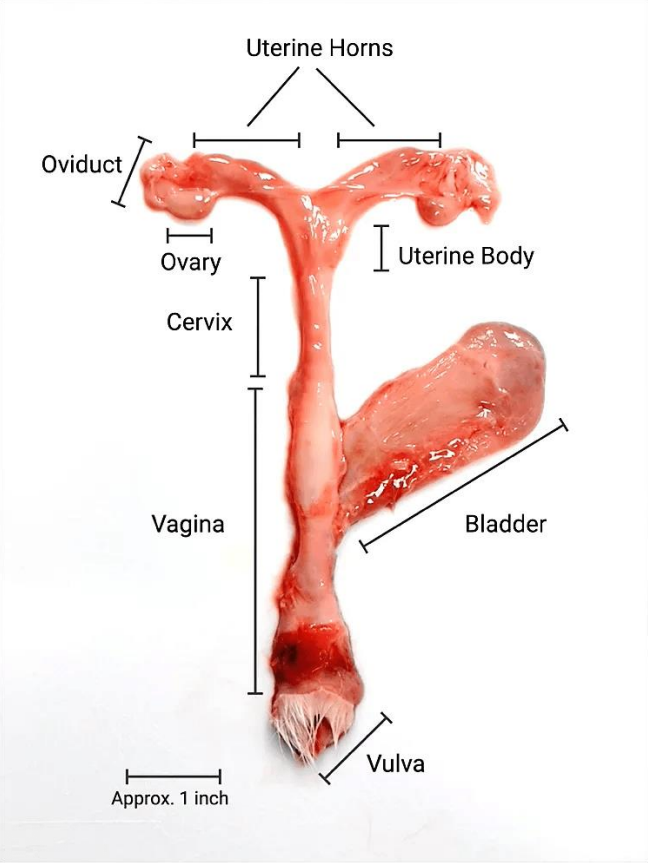

Figure 1. A 1-year-old doe's reproductive tract: the ovary, oviduct, left and right uterine horns, uterine body, cervix, vagina, vulva, and bladder.
Credit: Lillian G. Maxwell, UF College of Veterinary Medicine

If the doe is undergoing a laparoscopic insemination procedure, food is withheld on day 15 and water is withheld at least 8 hours before the insemination on day 16. Following removal of the CIDR, does must be inseminated within 56 hours. Figure 2 demonstrates the timeline of estrus synchronization in does.

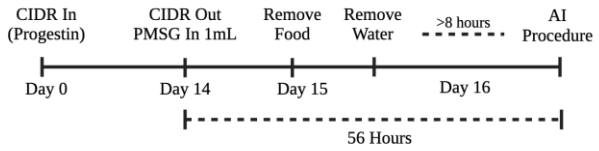

Figure 2. Timeline of most commonly used estrus synchronization in white-tailed deer. On days 15 and 16, food and water are only withheld from the animal if laparoscopic artificial insemination is being used. Credit: Lillian G. Maxwell, UF College of Veterinary Medicine

Vaginal Artificial Insemination

Vaginal artificial insemination is a common practice in deer; however, white-tailed deer have a complex cervical anatomy compared to other ruminant species. Technicians must be skilled and knowledgeable on proper procedures for cervid-specific AI. Despite cervix dilation during estrus, it is extremely difficult to penetrate the cervix into the uterus due to the presence of multiple cervical rings. Because of this, there are two approaches to vaginal insemination: transvaginal and transcervical insemination. During transvaginal insemination, an AI gun loaded with the semen sample is inserted into the vagina and guided into the opening of the cervix. A speculum can be used to help visualize the cervical anatomy. Upon reaching the cervix (Figure 1), the semen is then deposited at the opening. This method results in pregnancy less than 50% of the time. Comparatively, in transcervical insemination, the AI gun is inserted into the cervical opening once the cervix is visualized. The semen is then deposited into the caudal folds of the cervix. Generally, the AI gun cannot be advanced more than 1 cm into the cervix depending on the age of the animal, the number of previous pregnancies, and the extent of dilation of the cervix at the time of insertion. Do not attempt to push the inseminator farther into the cervix because this can result in reproductive injury or infertility. There are also special considerations for maiden and aged does. The cervix of a maiden doe does not dilate as much as a multiparous doe, which puts them at risk for injury when using a speculum or conducting transcervical insemination. Thus, semen should only be deposited in the vagina in maiden does. After the doe gives birth, the cervix loses collagen and stretches, changing the structure and allowing dilation to occur more easily. In aged does, cervical distortion is common, and transcervical insemination should be carefully performed with specific precaution not to injure the cervix by excessive force or movement of the AI gun. Transcervical insemination typically results in higher success rates up to 70%. However, the success of both vaginal insemination techniques is largely dependent on the skill of the technician, proper synchronization and estrus timing, the quality of sperm, and the health of the doe. Both methods require a high concentration of motile sperm, around 400 million for transvaginal insemination and 100 million to 200 million for transcervical insemination.

Laparoscopic Artificial Insemination

Laparoscopic AI (LAI) is a minimally invasive surgical procedure that deposits semen directly into one or both uterine horns. This procedure should be performed by a veterinarian. Before the procedure, the doe is placed under light sedation using an appropriate sedative agent or combination. Light sedation is used because the procedure only takes 5 to 10 minutes. Animals should return to normal almost immediately after reversal of the sedative medication. While the animal is under sedation, it is critical to move or turn the animal properly to reduce the risk of internal injury. Once fully sedated, the doe will be placed into a reclined position with the hindlimbs higher than the forelimbs, typically using a special LAI tilt table. The fore- and hindlimbs of the animal are restrained, and a cover, such as a towel, is placed over the animal's face and eyes to help keep the animal calm. The abdomen of the doe is then shaved and cleaned with an appropriate surgical scrub such as alternating betadine and isopropyl alcohol. Two incision sites are identified lateral to the midline and a small incision is made through the skin and fascia (a thin layer of connective tissue in between the skin and muscle) on one side of the animal. Two instruments known as a trocar and a cannula are inserted through the muscle. The trocar is removed, leaving the cannula in place. The laparoscope is then inserted into the abdomen through the cannula to identify the reproductive tract. After identification, another incision is made adjacent to the previously placed cannula, and another cannula-trocar set is inserted. The trocar is then removed and a loaded LAI gun, sometimes called a transcap, is inserted through the cannula. The transcap acts as sheath for the aspic, needle, and semen straw, allowing easy injection of the semen. When it is sheathed, this device can be used to manipulate the reproductive tract to identify injection sites. At this point, some veterinarians will evaluate the ovaries to ensure a follicle is present before insemination. Once an appropriate injection site is located, the needle is unsheathed and the semen is injected directly into the uterine horn using the transcap.

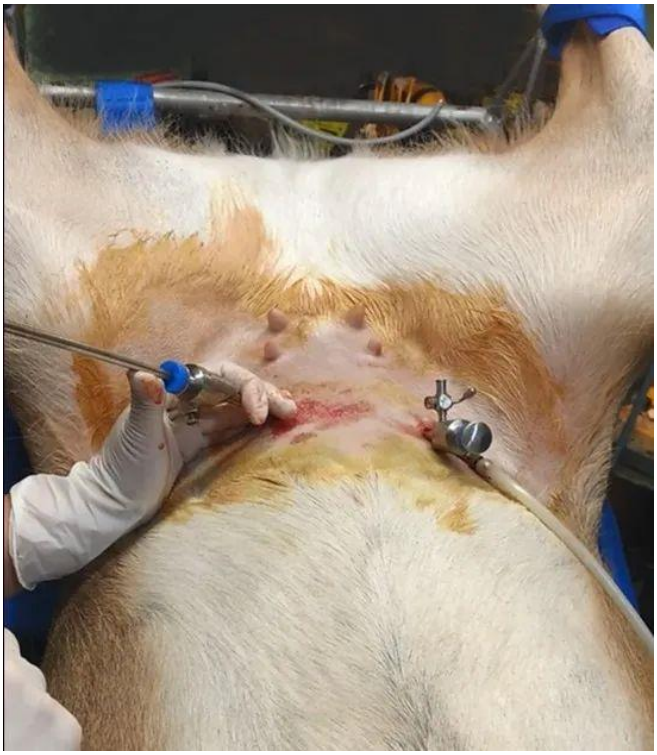

Figure 3. Site of laparoscopic insemination.
Credit: Juan M. Campos Krauer, UF/IFAS

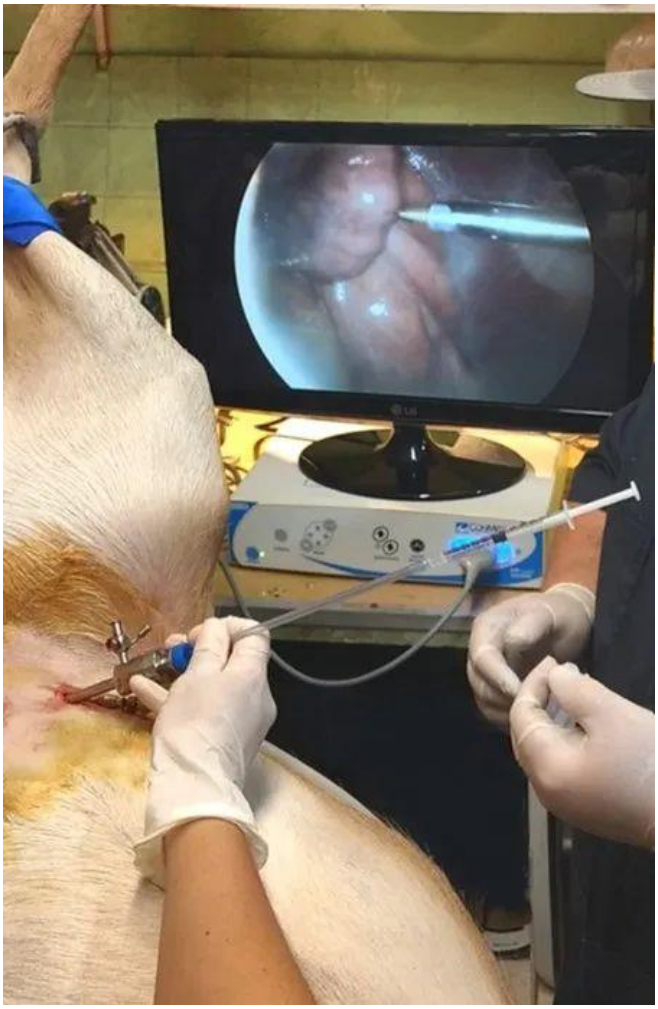

Figure 4. Telescopic view of semen being injected into the uterine horn.
Credit: Juan M. Campos Krauer, UF/IFAS

The LAI gun is then removed along with the laparoscope, and the incision is closed. It is possible to inject either one or both uterine horns. Typically, to inject one horn, a 0.25 cc semen straw is needed, but a 0.5 cc straw can be split between both horns. The LAI method has many advantages. LAI is considered the best insemination practice in cervids and has the highest success rate, around 60% to 80%. In the LAI procedure, lower concentrations of sperm can be used (20 million to 25 million motile sperm), allowing more dilute samples to be used. However, this procedure has disadvantages that include the cost of materials, the need for a skilled practitioner to perform it, and the evaluation of the semen before insemination.

Conclusion

When determining the right breeding procedure for your cervids, it is important to consider many things. First and foremost, managers should consider the safety, efficacy, and logistical constraints of each AI method. In place of AI, breeders can also opt to use natural breeding practices, placing does and bucks together during the breeding season. Approximately two to three weeks after AI, a buck can also be placed with does to increase likelihood of

insemination. This time interval allows managers to determine if insemination was successful. Each method of AI has a varying level of efficacy, but the best practice to increase conception rates is to work with trained professionals to conduct AI procedures. Other factors contributing to the success of AI include doe health and sperm quality. The University of Florida and the UF/IFAS Cervidae Health Research Initiative have extensive resources to promote the health of farmed deer. Consider contacting local UF/IFAS Extension agents, local veterinarians, or deer farming organizations for more information regarding deer AI practices or professionals in your area.

Additional Information

Mississippi State Deer Ecology and Management Lab: Biology of the Rut

Ask IFAS: "Tips for Successful Artificial Insemination in Beef Cattle"

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