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Prabuddh Dubey

PhD Scholar in Department of Veterinary Gynaecology & Obstetrics, COVAS, Department of Veterinary Gynaecology and obstetrics, College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University Agriculture & Technology, Meerut, Uttar Pradesh, India

Ashutosh Tripathi

Assistant Professor in Department Livestock Farm Complex- Gynaecology, COVAS, Department of Veterinary Gynaecology and obstetrics, College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University Agriculture & Technology, Meerut, Uttar Pradesh, India

Shubham Deep Chaudhary

PG Scholar in Department of Veterinary Gynaecology & Obstetrics, COVAS, Department of Veterinary Gynaecology and obstetrics, College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University Agriculture & Technology, Meerut, Uttar Pradesh, India

Garima Raj

PG Scholar in Department of Veterinary Gynaecology & Obstetrics, COVAS, Department of Veterinary Gynaecology and obstetrics, College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University Agriculture & Technology, Meerut, Uttar Pradesh, India

Corresponding Author:

Ashutosh Tripathi

Assistant Professor in Department Livestock Farm Complex- Gynaecology, COVAS, Department of Veterinary Gynaecology and obstetrics, College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University Agriculture & Technology, Meerut, Uttar Pradesh, India

Advances in artificial insemination in canines

Prabuddh Dubey, Ashutosh Tripathi, Shubham Deep Chaudhary and Garima Raj

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Abstract

Artificial insemination (AI) has become an important reproductive technology in canine breeding, enabling the efficient use of superior genetic material while overcoming geographical, behavioral, and physical breeding constraints. This review summarizes the principles, indications, semen collection methods, ovulation timing, semen preservation techniques, and insemination procedures in dogs. Accurate determination of ovulation, high-quality semen, proper semen preservation, and correct insemination techniques are essential for achieving high conception rates. Fresh semen provides conception rates comparable to natural mating, whereas chilled and frozen semen facilitate long-distance transport and long-term genetic conservation. Among insemination methods, vaginal insemination is commonly used with fresh semen, while intrauterine techniques are preferred for frozen semen to improve fertility. Advances in semen cryopreservation and standardized AI protocols have significantly enhanced breeding efficiency, contributing to the global dissemination of valuable canine genetics.

Keywords: Artificial insemination; Canine reproduction; Dog breeding; Semen cryopreservation

1. Introduction

Unlike female dogs, male canines are capable of reproduction year-round. A healthy, well-conditioned stud dog can breed every other day without any significant drop in sperm quality or production. With modern frozen semen technology, celebrated males can sire litters long after they have passed away. Driven by these benefits and a growing interest in dog breeding, artificial insemination (AI) has surged in global popularity.

The first documented successful AI in bitches was performed in 1784 by the Italian scientist L. Spallanzani. Since then, breeders have utilized fresh, liquid-stored, and frozen semen with varying levels of success.

- To achieve high conception rates, four key elements must be managed properly:
- Accurate identification of the ovulation day.
- Use of high-quality semen.
- Proper techniques for semen dilution and preservation.
- Correct execution of the insemination process.

Indications for Artificial Insemination

- Artificial insemination is commonly recommended in situations where natural mating is difficult, unsafe, or impractical:
- Physical Barriers: Vaginal strictures or a narrow vulva/vagina in virgin bitches.
- Behavioral Issues: Bitches that are overly dominant or submissive, or dogs that are overly timid or aggressive.
- Medical & Physical Limitations: Male health conditions preventing mounting, or spinal/hind limb pain in older stud dogs.
- Health & Logistics: Preventing the transmission of venereal diseases (such as brucellosis), recording precise semen quality metrics, or overcoming large geographical distances between the stud and the bitch.

Semen Collection

Manual manipulation to stimulate erection and ejaculation is the preferred method because it causes minimal stress to the male. It involves applying rhythmic pressure to the base of the dog's penis while collecting the fractionated ejaculate into a pre-warmed funnel attached to a collection tube. While electro-ejaculation under general anesthesia is used in wild canids, it is avoided in domestic dogs due to inferior ejaculate quality. Healthy studs can be collected every 1 to 2 days safely.

The Collection Process

According to Romagnoli (2002), the technician massages the *bulbus glandis* until a partial erection occurs, retracts the prepuce, and inserts an artificial vagina (latex cone) or continues manual massage with a gloved hand. When the dog lifts its hind leg, the penis is pushed backward and rotated 180 degrees.

Canine ejaculate is naturally expelled in three distinct fractions:

1. **First Fraction:** Prostatic fluid (only a few drops expelled during mounting attempts).
2. **Second Fraction:** Sperm-rich fraction (0.5 to 3.0 ml, varying by testicular volume).
3. **Third Fraction:** Prostatic fluid (4 to 50 ml, varying by prostatic volume).

Note: Fractions one and two are collected for general semen evaluation, while fractions one and two plus 1-2 ml of fraction three are used for AI. Excess prostatic fluid is typically discarded or excluded during freezing.

Determining Ovulation and Timing Insemination

On average, both proestrus and estrus last about 9 days each, with ovulation occurring 3 days after the onset of estrus (roughly 12 days after proestrus begins).

- **Day 1:** First day of visible bloody discharge (proestrus onset).
- **Day 10:** Average Luteinizing Hormone (LH) surge.
- **Day 12:** Ovulation occurs.
- **Day 14:** Ideal window for conception.

Diagnostic Monitoring

Because ovulation timing can vary widely (anywhere from 5 to 27 days after proestrus starts), precise tracking is essential:

- **Vaginal Smears & Behavior:** Performed every 2–3 days starting on day one. Estrus is confirmed when the bitch accepts the male or shows >70% vaginal cornification.
- **Progesterone Assays:** Blood tests show specific hormonal milestones: 2–3 ng/ml on the LH peak, 4–8 ng/ml at ovulation, and 10–25 ng/ml during the two days following ovulation when fertilization takes place.
- **Ultrasound:** Uses 5–7.5 MHz probes to track follicular growth and spot the disappearance of hypoechogenic areas (signaling luteinization) and fluid accumulation within the bursa.

Semen Storage: Fresh, Chilled, and Frozen

- **Fresh Semen:** Highly efficient, yielding conception rates >80% (comparable to natural mating).
- **Chilled/Liquid Semen:** Requires extenders to protect spermatozoa from temperature shifts, mechanical trauma, and pH imbalances. Milk-based extenders maintain motility for 24 to 36 hours, while Tris-fructose-egg yolk extenders can preserve refrigerated semen (5 °C) for 4 to

5 days.

- **Frozen Semen (Cryopreservation):** Enables global germplasm sharing and long-term storage, though post-thaw fertility rates are generally more variable and lower than fresh semen. Tris is the most common extender, and glycerol serves as the standard cryoprotectant. Thawing is typically performed in a 37 °C water bath for 15 seconds (for 0.5 ml pellets), though alternative fast-thawing protocols exist depending on the specific freezing method used.

Techniques of Artificial Insemination

1. Vaginal Insemination

The most common and straightforward method, highly successful when using fresh semen. Special equipment like the **Osiris pipette** (which features an inflatable balloon mimicking an erect canine penis) helps prevent backflow. Following deposition, the technician often "feathers" the vagina via digital stimulation and elevates the bitch's hindquarters for up to **15 minutes** to maximize conception rates.

2. Intrauterine Insemination (IUI)

Intrauterine deposition is widely considered mandatory for achieving optimal results with frozen semen. Several approaches include:

- **Transcervical Approach:** Performed using a Scandinavian or Norwegian catheter in a standing bitch. The cervix is stabilized manually through the abdominal wall, and the catheter is gently guided through the cervical canal into the corpus uteri. Whelping rates of up to 84.5% have been reported with frozen-thawed semen.
- **Endoscopic Insemination:** Utilizes a rigid fiberoptic cystourethroscope to visualize the external os cervix, allowing a urinary catheter to be safely guided into the uterus.
- **Laparoscopy:** A minimally invasive surgical approach that provides high conception success rates by directly visualizing the uterus.
- **Laparotomy:** A full abdominal surgical approach performed under general anesthesia where semen is injected directly into the uterine lumen. Though effective (~60% conception rates), it is the most invasive option.

Conclusion

Over the past two decades, artificial insemination has evolved into a well-established breeding practice. With standardized protocols—particularly for frozen semen management—AI now delivers conception rates comparable to natural mating. As a result, its acceptance among breeders continues to grow, fundamentally transforming the global dissemination of canine genetics.

Conflict of Interest

Not available

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