

EVALUATION OF ESTROUS DOG INFERTILITY INSEMINATION

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Artificial insemination (AI) is a common practice employed by breeders and veterinarians. In dogs, fresh semen (undiluted), chilled semen with added extender, or frozen semen may be used. Successful AI requires proper general husbandry, normal semen, a fertile female, understanding of the estrous cycle, and good semen placement technique.

General Husbandry

Collection supplies should be clean and spermicide-free. Some general household cleaning supplies may affect sperm viability and longevity so check with your veterinarian before using a non-approved cleanser for equipment. We suggest Ivory dish soap and distilled water.

Semen Evaluation

Collection of semen should terminate when the ejaculate becomes clear (this is the third fraction and is sperm-free prostatic fluid). If collection time is too short, there will not be enough sperm-rich fraction (second fraction). If collection time is too long and the third fraction is included, the semen will be dilute. Sperm motility is impacted by prostatic fluid in the test tube over time. Total semen volume should be 2-20 mls and volumes of each fraction collected should be noted. Generally, the sperm rich portion of the ejaculate is 0.25 to 3.0 mls. Semen should be evaluated for sperm morphology, quality of sperm motility, and the exact sperm count. Slides should be stained to assess sperm morphology. Parameters for normal semen are listed below:

- Morphology: <10% primary defects, < 20% secondary defects
- Motility: >70% of sperm should have progressive forward motility
- Sperm Count: >200 million sperm/ejaculate
- Cytology: No inflammatory cells
- Seminal Alkaline Phosphatase: > 5000 IU/L

Semen Handling

Fresh semen should be quickly evaluated for color, consistency, and normal number and motility of sperm. A warmed (body temperature) glass slide should be used for evaluation and the collected semen should be kept warm and out of ultraviolet light rays. The bitch should be inseminated within 5-10 minutes of collection. Success rates vary from 60-90% when proper AI with fresh semen has been performed.

A semen extender may be added to fresh semen with subsequent refrigeration to allow for use within several days.

Warming of the cold, extended semen must be done slowly and carefully to ensure viability of the sperm. Prior to insemination, a warmed drop of the semen should be evaluated for viable spermatozoa. Success rates with fresh extended semen with intravaginal insemination vary from 50-80%.

Extended semen may also be frozen for years however some sperm are damaged during the freezing/thawing process. Success rates with intravaginal insemination vary from 30-60% and 45-85% with intrauterine insemination (surgical or transcervical deposition).

Ovulation Timing

Insemination of the bitch at the correct time of her estrous cycle is critical for successful breeding. These are four methods for assessing the bitch's ovulation cycle:

Measuring progesterone levels: A small blood sample from the bitch taken every 1-2 days as she nears ovulation can be used to measure her progesterone levels. Ovulation occurs shortly (1-2 days) after an initial rise in progesterone levels. If a monitored dog has progesterone levels of less than 1ng/mL (baseline) and two days later increases to 2.0-3.0ng/mL we know that she is ovulating. She should then be mated every other day starting two days later.

Measuring LH levels: A brief surge in Luteinizing Hormone (LH) occurs right before ovulation. Measuring this hormone is a way of timing ovulation however because the surge is so brief (12-24 hours) it is easy to miss and progesterone levels monitored as well.

Vaginal cytology: Cells from the bitch's vaginal vault can be obtained with a cotton tipped applicator and examined on a glass slide. Under the influence of estrogen the vaginal cells change shape ("cornification") and the vaginal wall thickens and the in preparation for the "trauma" of copulation. When the majority of the vaginal cells collected are cornified (approximately 70%) one can use a direct hormone assay to determine the precise fertile period.

Vaginal endoscopy: A small fiberoptic scope can be used to visualize the inside of the vagina to look for morphologic changes associated with fertility. The inside surface of the vagina becomes more wrinkled as the bitch goes into estrus and is maximally wrinkled during the fertile period 4-7 days after the LH surge. Endoscopy should be used in conjunction with hormone assays to assess the most fertile period.

For optimal ovulation timing use blood progesterone levels, vaginal cytology, vaginal endoscopy, and LH levels to assess the bitch's full ovulation cycle.