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# Artificial Insemination, Using Fresh-Chilled, or Frozen Sperm

Thank you for your interest in ICSB-MA and Central Avenue Veterinary Clinic breeding services. In 1981, The American Kennel Club (AKC), recognized litters conceived from frozen semen, and subsequently, fresh chilled semen. This acceptance allows veterinary practitioners to assist clients with maximizing conception rates, using accepted techniques and methods. This packet will provide a better understanding of those techniques and methods.

# **Overview of Breeding Choices**

There are two basic choices for breeding canines: natural, or artificial insemination. The

Natural method, where the stud and the bitch "tie", uses no artificial means of insemination, but may result in conception, if the bitch's estrous cycle is known, and ovulation is anticipated.

Artificial insemination has three options, all using sperm collected from a stud dog and inserted into the female. Whether the sperm is fresh, fresh-chilled, for frozen, ICSB-MA offers in-clinic: Vaginal Insemination (A/I), Transcervical Insemination, (TCI) and Intra-Uterine Implant (Surgical Implant). Where these methods are used to inseminate, it is imperative that the estrous cycle be known, and the ovulation point known, to increase the prospect of pregnancy.

Being able to predict ovulation is of crucial importance when breeding by artificial insemination. To better predict ovulation, there are several methods, but the most commonly used for artificial insemination is Progesterone Testing (Prog Level). The importance of the testing hormonal levels, as well as other methods for anticipating ovulation will be discussed later in this article.

# **Semen Options for Breeding**

# Fresh-Chilled Semen:

Fresh-Chilled Semen refers to semen that is collected and shipped right away for use with a waiting bitch. This means that the female is at peak ovulation, or going to ovulate while the semen is in transit.

### Packaging Fresh-chilled Semen for Use

Preparation for shipping is an important step in the process of using fresh chilled semen. For this reason, care must be made to the method of packaging and shipping and includes:

- An insulated container (Styrofoam)
- Packing media (newspaper, or brown packing paper)
- Solid-frozen gel-ice packs
- Sturdy cardboard box that fits snugly around the insulated container
- The tube of the liquid semen sample, which should have "extender" in it

The method of shipping is also critical. Overnight deliveries are preferred. Packages flying in airplanes that are shipped on a non-pressured compartment of the airplane will cause the sample to become frozen, and rendered useless for breeding. Care must be taken to mark the package as a biological sample that MUST be placed in a pressurized area of the airplane.

### Preparation of the Fresh-Chilled Semen for Evaluation and Insemination:

ICSB-MA will receive the sample, and perform an evaluation. Only a small drop of sample is used for the evaluation, and the rest of the tube of semen remains refrigerated. The evaluation sample is viewed under a microscope to determine its characteristics. Those characteristics include color, viscosity, morphology, and motility.

ICSB-MA then determines the potential usability of the sperm, by re-evaluating the sample, according to ICSB methods. The determination of potential viability is also based on the client's preferred method of insemination. In some cases, a different method of inter-uterine insemination may be recommended, based on the veterinarian's disposition of the sample.

### Frozen Semen:

Frozen semen is semen that has been collected, evaluated, processed, and then stored in a in a nitrogen deep freeze (cryo-freeze), until it is ready to be used for insemination. Frozen semen ships in special metal containers, called "Tanks" or "Dry Shippers". The method of storage and shipment allows the semen sample to travel over distances and variations in ambient temperatures, without rendering it unusable.

This type of semen accommodates some degree of uncertainty about the expected ovulation date of the bitch to be bred, but demands that the sample be kept in a cryo-freeze the sample is ready to be evaluated and used for insemination. Aas long as the facility receiving the frozen semen is equipped with frozen nitrogen media and storage capabilities, receiving the semen a few days before the actual use presents no major problems.

#### **Evaluating Frozen Semen for use:**

Frozen semen that is going to be frozen for immediate or later use in artificial insemination is evaluated for certain characteristic that help ICSB-MA determine a potential viability for breeding. Those characteristics include: color, viscosity, motility, and morphology, number of spermatozoa. This is known as the pre-freeze evaluation. A second evaluation is performed when the frozen semen is going to be used.

#### Pellets vs. Straws:

ICSB-MA prefers frozen pellets that are stored in vials, and kept in a cryo-freeze. This technique of converting liquid semen to pellets that are cryo-froze was developed in the 1960s by Dr, Platz and Dr. Seager. There are samples that we receive, evaluate and use that are placed in "straws". In fact, we may have samples in storage at our facility in straw form because ICSB-MA received them in that form. Since the development of the pelletizing technique in the 1960s, ICSB-MA stores and processes frozen semen in pellets.

The preference for pellets has to do with the pre-breeding thaw: it is easier to confirm a semen's pre-thaw evaluation using a single pellet, than it is to thaw a potentially larger sample from a straw. Regardless of straw or vial, ICSB-MA does perform a prebreeding thaw to ensure that the semen is as usable for breeding, as it was at the time of initial pre-storage.

# **Methods of Artificial Insemination**

There are three methods widely used for canine breeding:

- A/I
- TCI
- Surgical Implant

### Artificial Insemination: A/I:

Sometimes referred to as vaginal insemination. Conception rates using this method rival those of natural breeding, when the proper technique and experienced veterinarians perform the procedure. The procedure is normally performed in a clinic setting.

This method of insemination should be performed at the time of ovulation, and is typically done twice within a particular period. Typcially, days 2 and 4, or days 1 and 3 post-ovulation.

This method is also used as a secondary breeding, after surgical procedures. Regardless of when the A/I is performed, progesterone levels should be known and should indicate that the bitch is within the range of ovulation.

### The Procedure:

Semen is drawn from a collection tube through an insemination rod. The size of the rod will vary by dog breed. The semen is deposited in the vaginal canal.

The Bitch is awake during the procedure and experiences minor discomfort, if any.

### Transcervical Insemination (TCI):

This method deposits collected semen at the entrance of the cervix, so that the semen can be drawn into the uterus. This procedure requires manual insertion of an transcervical scope and French catheter that contain a dispensing syringe. This procedure should happen only in sterile situations with an experienced veterinarian. As with all forms of artificial breeding, progesterone levels should be known and should indicate that the bitch is within the range of ovulation.

### The Procedure:

The bitch is positioned with her rear elevated, either manually, or with a breeding ramp, and carefully handled to avoid pressure on her abdomen.

Semen is drawn down into the syringe that is attached to the catheter. The catheter is then inserted into the transcervical scope. Once the entrance to the cervix is located, the catheter is inserted through the cervix into the uterus, and semen deposited. The bitch remains with her rear elevated for 6 minutes after the semen is deposited, to allow for gravitational feeding of the semen to the anterior vagina.

Post insemination, the bitch's activity should be restricted for 1 to 2 hours.

### Surgical Intra-Uterine Deposition of Semen (Surgical Implant):

Advances in this method have result in conception rates that are equal to those in natural breeding. Using frozen stored semen is more convenient than shipping bitches for breeding. Additionally, bitches that may have had conception problems relating anatomical barriers (size), or ovarian problems that made conception difficult, are known to be able to conceive using this method. Surgical Implants also tend to produce higher litter numbers.

Surgical implants typically are done 3 days post ovulation, and typically in sequence with other artificial insemination methods, like A/I or TCI following. As with all forms of artificial breeding, progesterone levels should be known and should indicate that the bitch is within the range of ovulation.

#### The Procedure:

The canine is anesthetized, and prepped for surgery.

A 4 cm to 6 cm incision is make midway between the pubis and the umbilicus and the uterus is identified and elevated to the surface incision.

Semen that has been specially prepared for the surgery is injected by a catheter and syringe attached to a 20 gauge needle. The semen is injected into the uterus and then dispensed.

The bitch must have her rear elevated post-surgery to ensure the deposited semen remains where placed.

The bitch should be given the appropriate time to awake, and is encouraged to have her activity restricted for several hours, post-surgery.

#### Acknowledgements:

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