

Maximizing Conception Rates

Dr. Robert Hutchison

Animal Clinic Northview, Inc.

34910 Center Ridge Rd.

N. Ridgeville, OH 44039

(440)327-8282

e-mail address repro@eriecoast.com

Since the American Kennel Club's recognition of litters conceived from frozen semen in 1981 and the subsequent acceptance of fresh chilled semen, practitioners are being asked more frequently to assist clients with maximizing conception rates. The gratification one feels when successful is one of the great rewards in veterinary medicine.

(SLIDE OF CERVIX) slide #1

The bitch is unique when compared to other domestic animals. The ova are ovulated in a progesterone "bath" as opposed to the estrogen environment of other species. The uterus is not easily accessible due to the cervix being difficult to reach, small in diameter and irregularly shaped (pointing caudal ventral into the vagina and cranial-dorsal into the uterus).

The bitch ovulates an immature egg that requires a further meiotic division before fertilization can occur. All this process takes place in an estrous cycle averaging 21 days of which the fertile period is approximately 72 hours.

Ovulation timing in the bitch has been attempted in many methods including:

- 1) Physiological Signs**
- 2) Estrogen Monitoring**
- 3) Lutenizing Hormonal Spike**

Physiological Signs: *Flagging*; softness of the vulva, and color of discharge have historically been used to determine the proper time to successfully breed a bitch. The fact that fresh semen lasts for a number of days in the bitch allows for “rough guesses” of ovulation to be successful in achieving conception. Multiple breedings also cover a wide range of time making conception likely.

The recent popularity of fresh-cooled extended semen, frozen semen and limited availability of desired males has made a more specific method of ovulation timing mandatory.
(slide of semen life)

Estrogen Monitoring: *The* use of estrogen for anticipating the ovulatory timing of the bitch is not reliable. The most frequently used method of estrogen monitoring has been through the use of vaginal smearing. By taking a cotton swab and sampling the cells lining the vaginal tract one can gauge the development of the ovarian follicles.

As the estrogen level rises, the vaginal wall thickens. As the serial samples are taken, one compares the cellular hydration, cytoplasmic/nuclei ratio and the staining of the chromatic material. As the estrogen level continues to rise, the vaginal wall will reach a 25-30 cell thickness. The cells dehydrate and become “cornified”.

The dehydration of the vaginal epithelial cells does not, however, reach a peak but rather stays in a greater than 80% cornified state for a period of 6-10 days. This does not equate with ovulation timing or indicate that ovulation has actually occurred. Many other methods of estrogen timing in the bitch have been tested, but due to the uniqueness of the bitch’s ovulatory process, estrogen is not an acceptable nor a consistent method of timing the bitch’s breeding pattern. (slide of vaginal smears)

Lutenizing Hormone (LH): *Lutenizing* hormone (*LH*) from the pituitary gland triggers the

release of the ova from the follicles. The release of the LH occurs for a 12-24 hour period.

Urinary testing for LH was unsuccessful due to the necessity of collecting the first morning urine from the bitch and the fact that the LH is metabolized into different fractions, some were detectable in the urine by commercial testing, other fractions were not.

The serum testing for lutenizing hormone has been used successfully to anticipate the release of the ova . An initial rise of progesterone from the ovarian follicle triggers the pituitary to release the LH. The short duration of the LH in the bloodstream requires daily testing which can be time consuming to the client, expensive, and uncomfortable to the patient. LH is species specific and levels cannot be confirmed by sending the serum to an outside lab. No laboratory in the United States is currently running canine LH on a commercial basis.

Progesterone Testing: *The* release of progesterone from the ovary and its subsequent rise to an average of 2 to 3 nanograms(ng.) signals lutenizing hormone release from the pituitary gland and denotes the start of the ovulatory process. The rise of progesterone to greater than 5 ng. (5 to 8 ng) indicates that ovulation has occurred. Once the ovulation day has been confirmed, insemination timing can be planned depending on the anticipated life of the semen being used.

The bitch needs to be tested every 48-72 hours to anticipate the prime breeding time. Progesterone rises and continues to stay elevated for approximately two months in the bitch, whether she is pregnant or non-pregnant.

The normal time interval between LH release and ovulation is 48 hours. Cortisol release needs to be a concern when doing progesterone testing as stressed bitches may have a delayed period between a rise of 2-3 ng and the time that the progesterone rises greater than 5 ng. Some bitches under stress have been shown to not ovulate even after a rise of progesterone above 2 ng. It is essential, when using progesterone testing, to confirm that the bitch has risen above 5 ng and has indeed ovulated.

Reported problems have made the in-office use of progesterone Elisa tests of questionable value. Hopefully these problems can be corrected and these valuable tests can be

returned to the veterinary laboratory.

Methods of Breeding

Natural: *If* the male is proven, local and available a natural breeding is the preferred method. Progesterone testing is still necessary to confirm that the breeding corresponds with the ovulation.

Vaginal Artificial Insemination: *If* timing is correct, but a natural is not possible nor desired, a vaginal insemination will enable the semen to be deposited at the external cervical opening. This method can also be applicable when a natural breeding can be a risk for disease, injury to the dogs or an inconvenience to the owner.

The drawback to a vaginal insemination is that the semen is deposited in the vagina and must be properly positioned for it to be drawn into the uterus so that the sperm cells can swim to the fallopian tubes (around the ovaries) where conception occurs. **(slide: bitch on ramp and vaginal insemination)**

When proper timing of the estrous cycle is performed and proper semen handling and delivery is accomplished, conception rates should rival those of natural breedings. Delivery technique problems such as improper placement of the insemination rod, improper semen placement, or semen damaged due to mishandling, have unfortunately convinced many dog breeders and veterinarians that artificial inseminations should be only used as a last ditch measure.

The bitch is positioned with her rear elevated either manually or on a breeding ramp. Care should be taken to avoid pressure on the bitch's abdomen. The semen is drawn from the collection tube through an insemination rod of proper length to reach the cervical opening. It is important that the semen be deposited at the entrance to the cervix so that the semen can be drawn into the uterus. With gloved hands, the veterinarian gently inserts the insemination rod through

the lips of the vulva at an upward 45 degree angle. The rod is gently passed over the pubis and along the dorsal median fold until it is parallel with the lumbar spine and localized in the area of the cervix. If resistance is encountered, the rod should be gently twisted or withdrawn a short distance, then advanced again.

When the insemination rod is properly positioned, the semen should be gently inseminated. The syringe is then removed from the rod. It is not necessary to push large amounts of air into the rod nor normal to get semen back flow if the rod is properly positioned and the bitch is in the proper stage of estrous. Excessive air “bubbling” through the semen is detrimental to the fragile plasma membrane of the head of the spermatozoa.

The bitch is then “feathered” digitally for one minute, the rear of the bitch is maintained in an elevated position for a few minutes to allow gravitational feeding of semen to the anterior vagina. The bitch owner is instructed to confine the bitch or restrict her activity for one to two hours post-insemination.

Intra-uterine deposition of semen

A technique for surgical insemination in the bitch was first described in 1974. The intra-uterine deposition was initially used to increase poor conception rates in the use of canine frozen semen. Since that time, deposition of semen into the uterus has become a routine technique used in numerous situations encountered in canine reproductive medicine, resulting in dramatically improved conception rates.

Situations where this can be helpful include:

A) Frozen Semen: Due to the lack of spermatozoa energy, buffer chemical makeup, or cervical resistance, the conception rates from cryo-preserved canine semen have been historically low when used with a vaginal insemination. Deposition into the uterine lumen has resulted in conception rates equal to those from natural breeding.

B) Fresh Cooled Extended Semen: Shipment of semen rather than shipping of the bitch has become common place. Due to shipment time and spermatozoa energy depletion, numerous clients have chosen intra-uterine semen insemination for their bitches in hopes of increasing conception rates.

C) Bitches with suspected uterine or ovarian disease: The ability to access uterine and ovarian health at breeding time is advantageous to clinicians presented with bitches with histories of reproductive failure. This can be accomplished doing a surgical insemination.

D) Giant and Toy Breeds: Individuals on the extreme ends of size have historically been recognized for conception difficulties. The ability to overcome anatomical barriers and inseminate directly into the uterus has increased litter numbers and relieved client frustration.

E) Males with lowered or compromised spermatozoa numbers: Little work has been done in the canine to definitively define minimum semen parameters necessary for conception. By directly depositing the semen into the uterus and bypassing the cervix and vaginal vault, conception can be achieved with lesser sperm numbers and lesser overall semen quality.

Introducing the semen into the uterus can be accomplished using two different methods.

1) Trancervical Insemination:

Trancervical Insemination (*TCI*): This technique entails the use of a cysto-urethroscope using fiberoptics to visualize the external opening of the cervix. A flexible catheter is maneuvered through the external cervical opening and subsequently into the uterus. **(picture of Dr. Hutchison doing TCI)**

The TCI requires no anesthesia and minimal restrictions for the bitch. This technique allows the semen to be deposited into the uterus but does not allow visualization of the uterine lining by the veterinarian. This technique is most useful with compromised semen life, but a suspected normal uterus in the bitch. The technique has shown to not be useful for endometrial biopsy.

2) Surgical Intrauterine Deposition of Semen

A pre-surgical evaluation is performed on the bitch. Anesthetic induction consists of coning smaller individuals with gas anesthesia (i.e. Isoflurane, Sevethane). Larger bitches may require an injection of a standard, short acting anesthetic (i.e. Propofol) to achieve relaxation for intubation and maintenance with gas anesthesia.

The bitch is prepared in the same manner as a bitch undergoing a ovario-hysterectomy. The ventral abdomen is clipped and the bitch is placed in the surgical theater in dorsal recumbency. Prepping of the surgical site is done in a routine manner. The abdomen is draped in preparation for the surgery.

A 4 to 6 centimeter incision is made midway between the pubis and the umbilicus. The incision is made in the skin, subcutaneous fat and through the linea alba. The uterus is identified and elevated to the surface through the incision. The uterus is draped with a saline moistened laparotomy pad as the semen is prepared for the injection procedure.

A volume of semen varying between .5ml and 4ml is prepared for insemination. If the volume is greater than 4ml, the semen should be centrifuged for five minutes. The supernatant is decanted and disposed. The semen pellet is gently re-suspended with the remaining supernate or with a semen extender.

The semen to be injected is gently drawn into a 6ml syringe through an insemination rod. The rod is removed and a 22-gauge 3/4-inch needle is attached to the syringe. **(picture of surgical insemination of semen)**

The surgeon inserts the needle into the lumen of the uterine body at a 45-degree angle with the bevel of the needle up. The semen is slowly injected into the uterus. The semen should flow easily with obvious distention of the uterine horns. If the injection cannot be achieved or is difficult, the needle should be repositioned.

Saline moistened gauze is held over the injection site after the needle is withdrawn. After one minute, the gauze is removed and the uterus is replaced into the abdomen. Closure of the

fascia muscle, subcutaneous tissue and skin is the routine procedure.

The bitch's rear is elevated as she recovers from the anesthesia. The intra-uterine pressure during the surgical deposition of the semen may cause mild back flow through the cervix into the vaginal cavity. This appears to be of no concern. If the surgeon has doubt as to the semen being placed in the uterine lumen, a post-operative vaginal smear will confirm spermatozoa, if the technique has been performed properly.