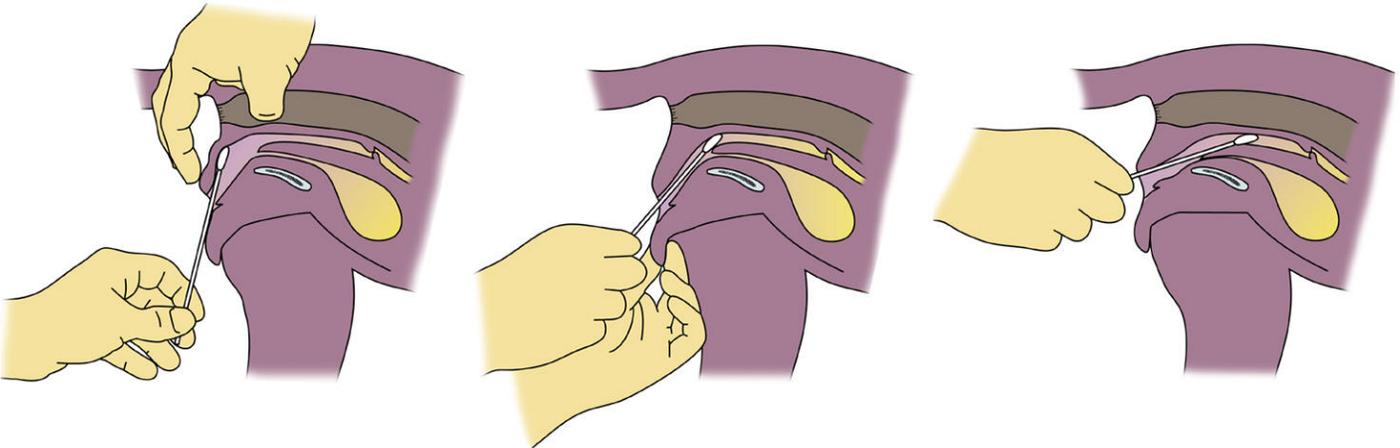


# Normal Cycle

## Canine Vaginal Cytology

### *Technique*

- Moisten a cotton swab with 1 to 2 drops sterile saline. Open the vulvar lips, pull the vulva dorsally, insert the swab dorsally and posterior, then up and over the pelvic brim and into anterior vagina. If you do not pass the swab far enough, you will get vestibular cells and result in false cornification. If you pass the swab too ventral, you may enter the bladder and get a falsely non-cornified smear.
- Roll the swab firmly onto a slide.
- Stain the slide using DifQuik stain, 10 dips in A, 15 dips in B, and 20 dips in C. You may also use new methylene blue stain.
- Read the slide under low power first to establish the trend of cellularity and cell types. Move to a higher power to establish the cell types. View several fields to get an overall visual idea of the percentage of cornified cells.



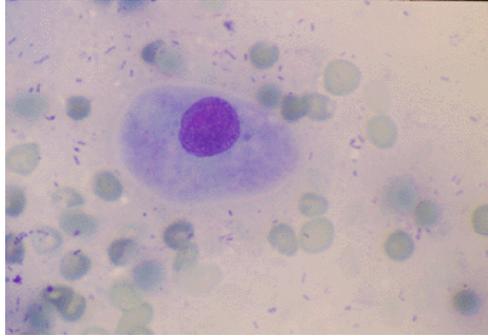
### *Vaginal Cytology Cell Types*

#### **Non-cornified**

**Parabasal cells have a large stippled nucleus and a rounded cytoplasm. The nucleus is large compared to the cytoplasm.**

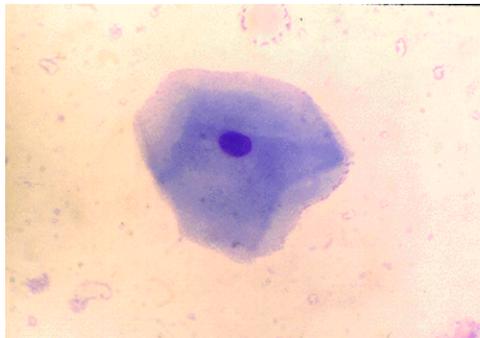


Intermediate cells have a have a stippled nucleus and more cytoplasm than parabasal cells.  
The cytoplasm may even become angular.

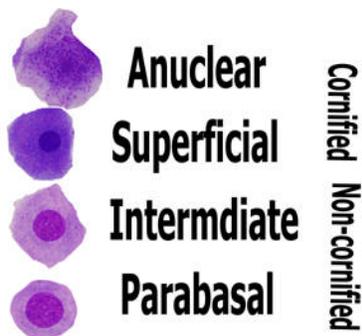
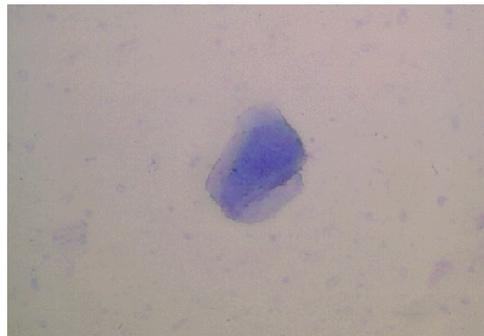


**Cornified**

Superficial cells have a pyknotic nucleus and angular cytoplasm.  
There is no stipling in the nucleus.



Anuclear cells have no visible nucleus and angular cytoplasm



## **CHILLED SEMEN INSEMINATION**

### **FRESH CHILLED SEMEN**

Fresh chilled semen uses energy as it is cooled to 40°F (4°C) and eventually re-warmed to body temperature. The life in-utero of spermatozoa having experienced the chilling and subsequent warming process is 24 to 72 hours, necessitating a more precise manner of ovulation timing and breeding. When a veterinarian receives a fresh chilled sample, the package should immediately be opened. Attention should be paid to the "impression of coolness". The ice packs should be at least cold, if not still frozen. The package containing the semen should be removed from the packaging material. The tube should contain the extended semen in a liquid state.

Unfortunately, occasional mishandling by the shipping company or by the shipper placing the semen package in a non-pressurized compartment of the airplane will cause the sample to arrive frozen. The freezing kills the sperm cell and renders the sample useless.

One drop of the sample should be placed on a warmed microscope slide. The rest of the sample should be refrigerated. Allowing the chilled sample to warm to room temperature only allows the sperm cells to speed up, using precious energy and shortening their life span.

In many cases, the semen will appear to be non-motile on the slide. However, as the semen drop warms gradually, side to side motility becomes noted. The continued warming eventually shows the cells to have achieved a normal forward progression. Do not warm the sample too fast. Micro-scope lights will over heat the semen and kill the sperm. If no motility is noted after fifteen minutes, the sample is most likely non-viable.

It is recommended that the refrigerated fresh chilled sample NOT be warmed to room temperature or body temperature before insemination. Having the sample in the uterus as it warms makes maximum use of the conserved energy. All fresh chilled semen samples are handled in a similar manner.

### **PROCEDURE**

The estrous cycle for the bitch should be monitored using serum progesterone assaying or some other reliable manner to ascertain that ovulation has occurred. Semen motility is evaluated as previously described.

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 placed into a large syringe from the collection tube. The syringe is then placed on an insemination rod of proper length to reach the cervical os. 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 re-advanced.

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 is it normal to get semen backflow if the rod is properly positioned and the bitch is in the estrus stage of estrous. Excess 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 fifteen minutes to allow gravitational feeding of semen to the anterior vagina. The bitch owner is instructed to confine the bitch or restrict her activity, which includes elimination for two hours post-insemination.

## ARTIFICIAL INSEMINATION IN A DOG

### A Surgical Approach To Breeding

Artificial insemination in the dog: Modern dog and cat breeding practices often include the practice of Artificial Insemination. Presented on this page is an example of surgical insemination directly into the uterus of a dog. Another technique that can be used that does not involve surgery is the trans cervical insemination. This new procedure allows semen to be deposited directly into the cervix without anesthesia. Special training is needed with the trans cervical technique in order to be certain that the semen is deposited within the uterus; this is done using an endoscope. Some of the obvious reasons for utilizing AI (Artificial Insemination) are the following:

You and your veterinarian will want to discuss the pros and cons of AI well before the predicted time the female will come into heat. Proper Prior Planning Prevents Poor Performance! Do lots of homework so that you know what is "out there", where you can get it, how much it costs to acquire the frozen sperm, and how your veterinarian will inseminate.

One method of insemination is to infuse the semen and associated nutrient fluids directly into the deep vaginal area of the bitch. The bitch then will have her rear quarters raised for a few minutes in an attempt to have gravity assist with the dispersal of the semen as deep into the vaginal area and as close to the cervical opening as possible. Then the bitch needs to remain confined for 6 to 8 hours after the insemination. The individual sperm cells need to pass through the cervix from the vagina and into the body of the uterus. From there, they disperse further up the two "Horns" of the uterus. The uterine horns are long hollow tubes, lined by the richly vascular endometrium, and extend from the body of the uterus upward almost to each kidney. At the end of these two tubes the ovaries are situated such that when they produce eggs, the eggs travel through a tiny opening into the far end of each uterine horn. (Just another wonder of nature!) If the eggs encounter sperm cells in their travels DOWN the uterine horns, there is a chance that the egg will be fertilized by some lucky sperm cell going UP the uterine canal and then another whole series of miracles take place that just might end up as a puppy!



Whether your veterinarian chooses to do the vaginal insemination or chooses to surgically implant the sperm directly into the body of the uterus is a matter for discussion well prior to the time a decision MUST be made. The following photos will take you through the surgical procedure. From start to finish, the surgical Artificial Insemination procedure takes only a few minutes. Light general anesthesia is required. Absolute sterile procedure is an important factor. The insemination fluid must be kept warm and implanted within the uterus as soon as possible after collection from a male who is present, or soon after shipped, frozen semen is thawed.