Canine Vaginal Cytology to Aid Your Breeding Successes

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Using Vaginal Cytology to Assist Your Breeding Program

Vaginal Cytology can be used to:

1. determine when to start running progesterone tests. Use this tool to save money on progesterone testing.
2. determine if there is reproductive tract pathology.

Prior to the ability to run progesterone tests in the veterinary clinic or kennel, the only timing we had for when to breed bitches was based on vaginal cytology. We as a community got VERY skilled at using this tool. However, it was also before we used much frozen or fresh chilled semen and before we could predict when to perform scheduled c-sections. The value of progesterone testing for timing frozen semen breedings, to manage male and female dogs with infertility, and to schedule c-sections for high-risk pregnancies cannot be overstated.

Now it is time for the pendulum to swing back a bit. Although progesterone testing can be convenient, have a short turn-around time, and is cost-effective, we should re-examine the value of vaginal cytologies. We can use this tool to minimize the number of blood draws and progesterone tests needed. It can also be to assess if the bitch has an infection in her reproductive tract that needs to be managed to maximize her chances of a successful pregnancy.

There are 5 stages of the heat cycle known as estrous. These are anestrus, proestrus, estrus (without the o means the ovulatory part of the estrous cycle), diestrus and metestrus.

Understanding the estrous cycle of the bitch:

To understand how to use vaginal cytologies and progesterone results to achieve a successful pregnancy, we need to understand a few things about what makes the bitch unique. Dogs and other canines are the only species that does NOT ovulate an egg ready to fertilize. Bitches need 2 to 3 days after ovulation to have their eggs mature enough to be fertilized.

Timing the breeding:

Mating Behavior – can be highly variable. Some females are receptive during their entire heat cycle, some only during their fertile period and some are never receptive to the male you have selected, or any male at any time. The same holds true for males – some are always willing to breed and others are only willing to breed certain females or at specific times. Some will be too shy to show mating behaviors in front of people or in front of certain dogs that have been dominant to them.

Most experienced males will wait until a female is "prime" to approach and mate. Young males may be too willing and others may be reluctant to be assertive. Sometimes a particular female may have previously told a male to back off so when she is ready, he is still wary. Older males may have joint, back, or prostate pain and be unwilling or unable to mount a female, even when prime, without medication for pain, arthritis or an enlarged prostate. For these males, appropriate medication can be helpful.

Artificial inseminations, in the form of side-by-side AIs, AIs at the vet, Transcervical inseminations (TCI) at the vet, and surgical AIs in special circumstances can overcome unwilling mating behaviors for both males and females. These are only recommended with good timing information with progesterone testing so you maximize your efforts. Putting good semen into a bitch at an infertile part of her cycle is not productive.

Vaginal cytology to determine when to start progesterone testing:

Vaginal cytology – is an old but useful tool. Vaginal cytologies will not replace progesterone testing for sophisticated breedings using fresh chilled or frozen semen. Nor is this tool accurate enough to time a scheduled c-section. However, if you are struggling with

timing, not getting females pregnant, or spending too much money on progesterone testing, this is a step toward better understanding the phase of a heat cycle.

This is something most veterinary clinics can easily perform for you. It is also a skill most breeders can learn with a little practice and the purchase of a microscope, cotton applicators (long Q-tips), microscope slides, and a stain kit called Dif-Quick.

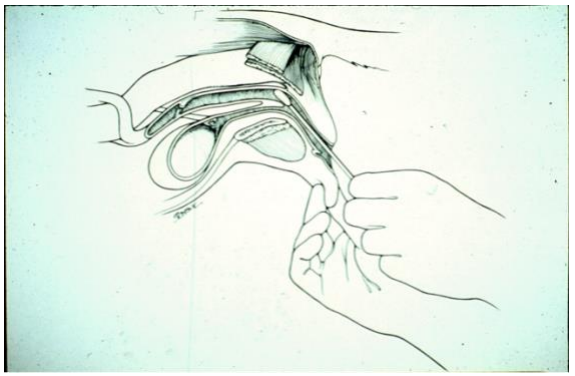
Photographs of the epithelial, red and white blood cells and bacteria will follow the text. Videos are available in the Learning Center on the Revival Animal Health Website.

The cells lining the vagina during the non-ovulatory phase of the bitch's cycle are very characteristic and easy to identify as "non-cornifed" or "parabasal" cells. As the bitch comes under the influence of estrogen, red blood cells appear and the epithelial cells change from non-cornified to cornified, in a stage of the heat cycle called proestrus. The closer to ovulation or estrus she comes, the higher percentage of cornified cells are seen. At ovulation, when the progesterone rises, these epithelial cells approach 100% cornification and begin to clump or sheet together, making thick groups of cells on the glass microscope slide. Additionally, the red blood cells become fewer and may disappear altogether. As the bitch leaves her fertile period, usually 3 to 5 days after ovulation, these cells again round back up, regain their nuclei, become more separate on the slide, and again return to a non-cornified state. Additionally, during this time when she leaves estrus and enters diestrus and metestrus, white blood cells appear. During her entire cycle, bacteria in a variety of shapes (rods and cocci) may be present. This "mixed bag" of bacteria is considered normal and do not justify the use of antibiotics. If white blood cells are seen on the slide during proestrus, this is an inflammatory cytology and should be discussed with your veterinarian.

Why should you use vaginal cytologies?

1. To determine when to start running progesterone tests. A vaginal cytology, after you have purchased a microscope, literally costs pennies to run, compared to the cost to run a progesterone. These do not require a blood draw, centrifugation, and testing. Once she starts to show cornified cells, it is time to start running blood tests.

2. To determine if she has pathology or an infection, in her reproductive tract.
   If white blood cells are noted on the cytology prior to ovulation or cornification of the cells, a culture and/or an antibiotic MAY be justified. Routine use of antibiotics during each heat cycle increases the risk of the bitch developing resistant bacterial infections. This means later on, if you need antibiotics for her, other dogs in the kennel or even the people in your household, the bacteria are likely to not respond to antibiotics, leading to unmanageable bacterial infections such as MRSAs.

   If you are doing vaginal cytologies in the kennel, you may find it helpful to take these samples to your veterinary clinic for them to evaluate. Together, you and your favorite veterinary technician can learn to lean on vaginal cytology.

What supplies do you need to do vaginal cytology?

1. Microscope, if possible one with a screen so the slides can be looked at without using the oculars of the microscope. This way, more than 1 person at a time can view the slide and identify cells and cell types.
2. Microscope slides.
3. A 3 step stain kit.
4. Cotton applicators or brushes – 6 inch long Q-tips with plastic not wooden handles or shafts. Wooden applicator sticks may break off in the vagina.
5. Saline to moisten the swab – do not use lubricant gels.
6. A bit of practice. Ask your clinic's veterinary team if they can help you learn to use the microscope and interpret slides.

How do you perform and interpret a vaginal cytology?

1. Stand the bitch in heat on a table or platform. Do NOT attempt this if she is not in heat.

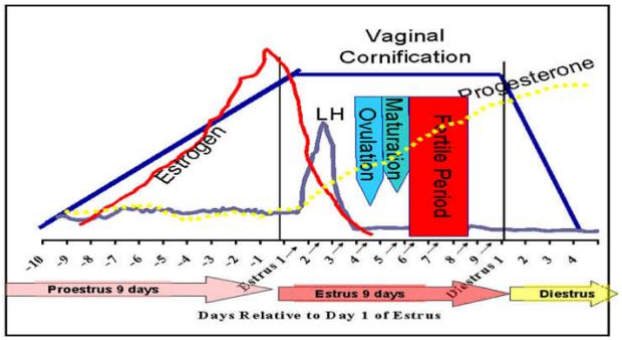
2. Have someone hold the bitch so she remains standing and does not turn around to nibble on you when you insert the swab.
3. Direct the swab through the lips of the vagina at a 45 degree upward angle toward the base of the tail, as you would to introduce a pipette to insert semen.
4. When the swab is inserted 4 to 6 inches into the vagina, gently roll the swab inside the vaginal vault.
5. Remove the swab.
6. Immediately, roll the swab on a dry new glass microscope slide. This must be done while the swab is still moist from the vaginal secretions. Roll, do not drag the swab across the slide.
7. Let the slide dry. Label the slide with the bitch's name and date the sample was collected so you can use for reference in the future.
8. Once the slide has dried, dip the slide into the fix, then the 2 stains according to the directions on the stain package. After dipping in all 3 solutions, turn the slide over and allow tap water to gently run across the slide surface that did NOT have the sample rolled onto it. Set the slide at an angle on a towel and allow it to air dry.
9. Once dry, place the slide on the microscope stage with the light turned on and on the lowest power. Focus on the cells in an area with a single layer of cells.
10. Examine 100 epithelial cells for
    a. The shape of the cell – is it lightly stained and round or is it darker and more angular on the edges?
    b. The presence of a nucleus – the dark purple center of the cell. Non-cornified or parabasal cells have a nucleus that is dark purple spot in the center of the cell. These cells look like fried eggs, with round edges, with the nucleus of the cell looking like a purple egg yolk. When cornified, the nucleus will disappear, and the edges of the cells will become jagged and irregular.
    c. Are the epithelial cells laid out singly on the slide or are they in clumps or sheets? Single cornified cells indicate early estrus, and clumps or sheets of cells indicate she is ovulating.
    d. Examine the slide for red blood cells, white blood cells and bacteria. Red blood cells will be a light purple ghost-like cells with pale color and no nucleus, smaller than the epithelial cells, and have no interior structure. White blood

cells will have a light colored outer area and a darker purple bean shaped center. Bacteria will be very small compared to red and white blood cells. They may be round (cocci) or elongated (rods) in shape. Bacteria are normal.

e. Estimate or count the number of cornified versus non-cornified cells. In the early part of the estrous cycle, known as proestrus, you will see red blood cells, bacteria, and non-cornified or parabasal cells, the ones that look like fried eggs, at 100%. As her estrogen rises, drops, and the progesterone rises from baseline (< 2 ng/ml), the cells begin to stain more darkly, look less round and more angular, and lose their nuclei. When she approaches ovulation (progesterone 5 ng/ml and higher), the % of cornified dark staining, jagged edged, non-nucleated cells increases and the number of red blood cells decreases. Some bitches achieve 100% of the cells becoming cornified as the progesterone rises while others may not rise about 75%.
f. As the cells become 50% or higher cornified, start running progesterone blood tests. The ideal day to breed if you are only breeding one time during the heat cycle is when the first cells drop from 100% cornified to becoming more rounded up and regain their nuclei. If you are not watching closely, you may wait too long. If you are doing multiple breedings, begin to breed with the cornified (potato chip cells with no nuclei) exceed 75% of the epithelial cell type.
g. If you do a cytology and the cells are all non-cornified or parabasal, there are white blood cells, and the progesterone is above 10 ng/dl, it is likely too late to breed on this cycle and expect a normal sized litter.
h. Save and catalog the slides you have collected for later reference. These can be useful in assessing why a bitch missed on a breeding, what the bitch's last heat cycle was like, for practice, and to teach others how to interpret vaginal cytologies.

How does progesterone work?

Progesterone – one female hormone used to accurately time when to breed and to time when the pregnancy should end.



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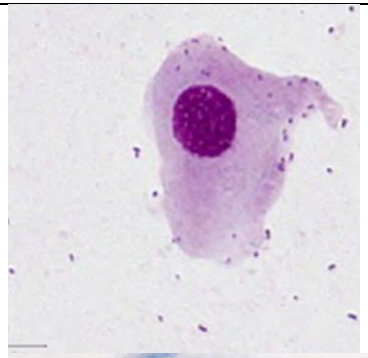
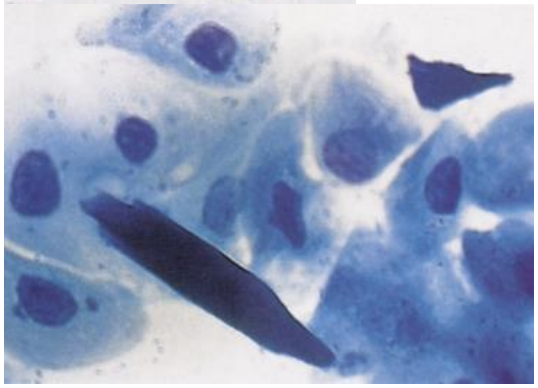
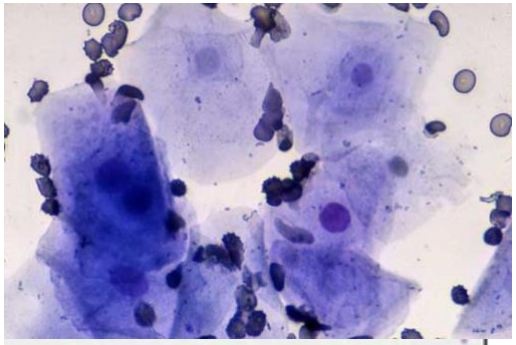
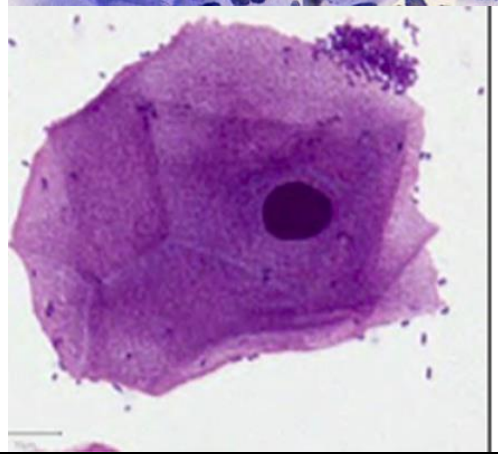
Quantitative progesterone tests can be run in-house or sent to reference labs. Semi-quantitative tests – run in wells with visual color changes are not accurate enough to time bitches for frozen semen breedings or to time them for c-sections. There are at least 6 machines that can be used to run quantitative tests in-house – that is a test that provides a specific number, not an estimate based on a visual color change.

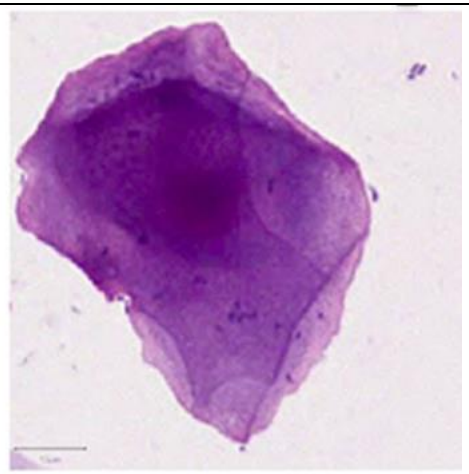
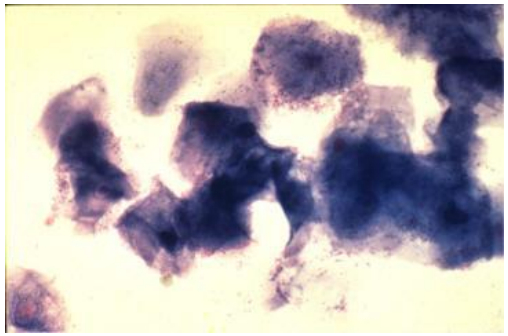
Progesterone starts low – less than 1.0 ng/dl when a bitch comes into heat. It will rise, sometimes with a wobble, as her heat progresses. Ovulation occurs when the progesterone rises to 4 ng/dl to 8 ng/dl. As her heat cycle progresses, progesterone will rise to as high as the mid 40s and stay high during the pregnancy. As whelping approaches, the progesterone will slowly drop.

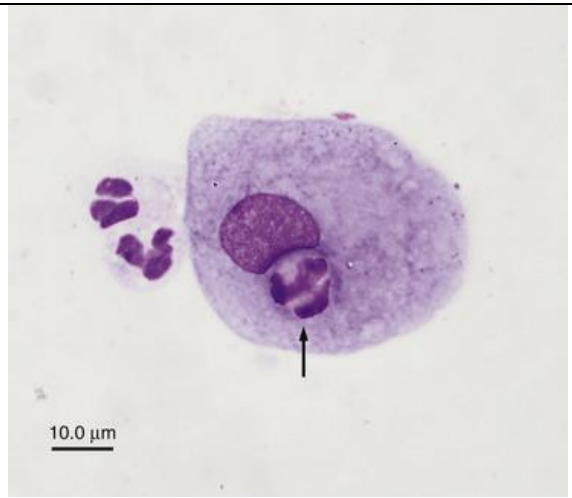
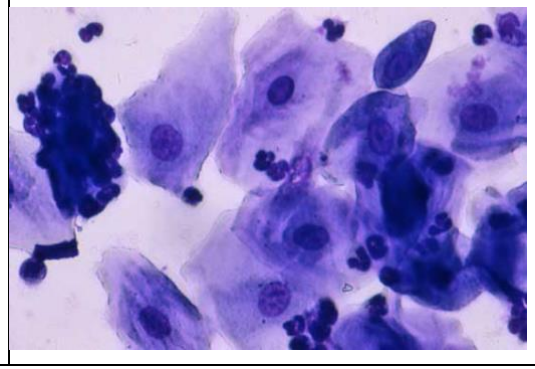
For timed breedings, fresh semen or natural breedings should occur 2 days after the progesterone reached 4 to 8 ng/dl if the progesterone is between 8 and 16 ng/dl. For frozen semen breedings, the breedings, whether done by TCI (transcervical insemination) or surgical breeding, should be done 3 days after the progesterone reaches 4 to 8 ng/dl if it is over 20 ng/dl.

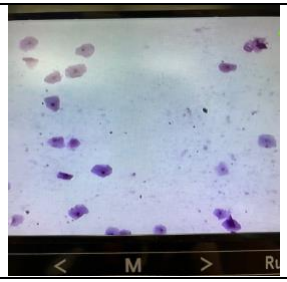
A bitch should whelp 63 days after ovulation (progesterone between 4 and 8 ng/dl) plus or minus 24 hours. For timed c-sections, it is safe to deliver the pups 62 days after ovulation. For timed c-sections on brachycephalic breeds (English Bulldogs, Pugs, French Bulldogs, and so on), it is safe to deliver the pups 61 days after ovulation (progesterone of 4 to 8 ng/dl). If adequate timing was not done at the time of ovulation and/or breeding, it is safe to deliver the pups when the progesterone drops below 2 ng/dl IF she does not have pre-term labor (premature delivery).

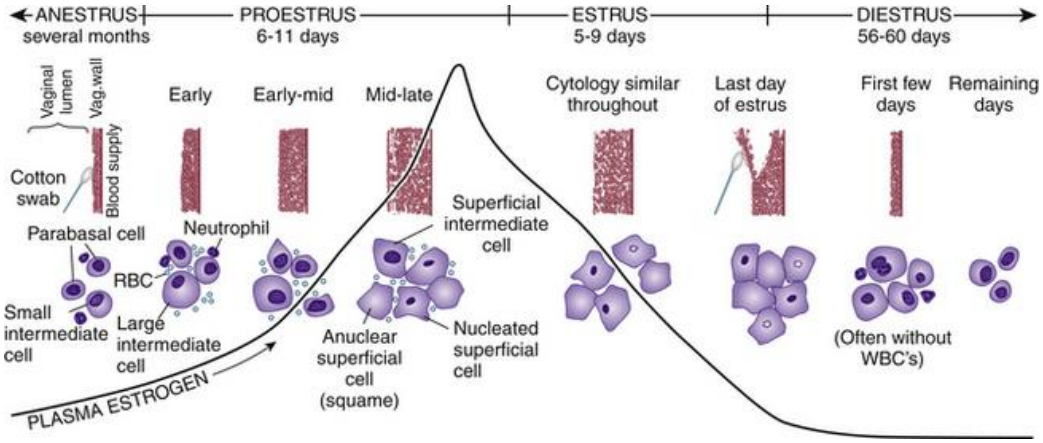
Stage of Heat	***Definition & Time Line***	***Progesterone***	***Cell Type***
Anestrus	The time until puberty and the 4 + months between heat cycles.	***Progesterone below 1.0 ng/dl*** In anestrus, there is little ovarian activity. The female will not have any swelling of her vulva and no vaginal discharge. In this stage, you will see bacteria and large non-cornified vaginal epithelial cells that are lightly stained with a dark purple central nucleus. ***Some bitches keep themselves very clean so seeing the 1st drop of blood may not be the start of her het cycle. Start running vaginal cytologies but wait to do a progesterone test when you see the cells change from round with a nucleus to angular, dark staining and losing their nuclei.***	 

Proestrus	The 1st 7 to 10 days of the heat cycle. Identified by swelling of the lips of the vulva and a bloody vaginal discharge	***Progesterone between 1.0 and 4.0 ng/dl,*** with small rises, and sometimes small drops. In proestrus, her estrogen levels are rising. There should be vaginal swelling and a bloody vaginal discharge. You will see bacteria of mixed types, red blood cells, and large non-cornified vaginal epithelial cells that are lightly stained with a dark purple central nucleus. Red blood cells will stain a light gray color, be smaller than the epithelial cells, and have a ghost-like appearance. ***As the cells begin to cornify, it is now the time to start running progesterone blood tests. A cornified cell is dark staining, angular, and some are losing their nuclei.***	 

Estrus	The fertile period of the heat cycle when the female is receptive, has ovulated and can conceive a litter.	***Progesterone 4 to 20+ ng/dl.*** ***Progesterone will stay high whether or not she was bred or pregnant.*** In estrus, her estrogen levels are dropping, she has an LH surge, and her progesterone level is triggered to rise. The vulva should still be swollen but is softening. The vaginal discharge will usually change to a straw color, although there are some females that have bright red blood discharge through the entire cycle. You will see some mixed bacteria, fewer to no red blood cells, and the epithelial cells will become larger, angular instead of round, dark staining, and fewer to none with a dark purple central nucleus. These cells will look like potato chips. When she is at her peak of fertility, the cells will be clumped together. ***If you are going to time for a fresh chilled or frozen semen breeding or to manage a high-risk pregnancy including a timed c-section, run progesterones now!***	 

Diestrus	The termination of the fertile period, at which time white blood cells and non-cornified cells appear/reappear	*Progesterone is usually above 20ng/dl but any value above 3 ng/dl may be normal.* *As she leaves diestrus and enters metestrus, her progesterone value will slowly degrade.* In diestrus and metestrus, her progesterone level will rise and stay high for 63 days, whether pregnant or not. Her vulva will return to a smaller size and there should be little to no discharge. Around 30 days after breeding, she may have a small amount of clear mucus discharge. You will see no red blood cells, fewer bacteria. In the place of red blood cells, you will see white blood cells, small cells with a c-shaped central nucleus. The epithelial cells will return to round smooth, light staining epithelial cells with a dark staining central nucleus.	 

Metestrus		*Progesterone will drop again to baseline, < 1.0 ng/dl*	



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