VET CHAT

Ovulation Timing

The Fertile Period in the Bitch

Dr Robert A. Zammit | Members and Friends Forum |

INTRODUCTION

Ovulation timing in the bitch remains an impossible parameter to be accurately defined in any female canine in oestrus. Worse still, is the absolute accurate definition of the "fertile period" within this species.

Nevertheless, ovulation timing and assessing the fertile period from the predicted day of ova release, remain an essential tool to successful conception, especially when using frozen semen. In total practical terms, the most essential element is determining the fertile period because this is when semen must be in the bitch's reproductive tract to effect fertilization and therefore, successful conception.
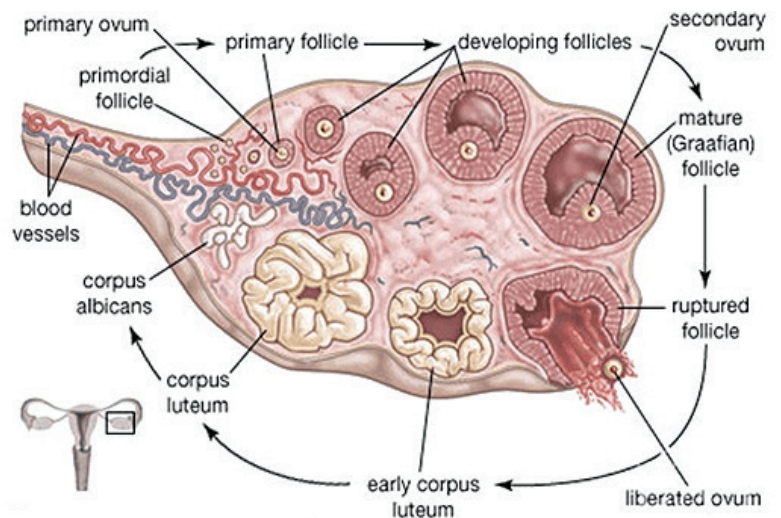
In the past, this was determined by such methods as the ever reliable stud dog, with breeders stating, "He always knows when they are ready." Or by counting from the first day of bleeding then mating on day 11 and 13. Other methods that have been developed involved watching for when the bleeding eased then mating 24 to 48hours later. Then it became more technical with breeders attempting to find a change in the glucose level of the vagina, others using a machine that pleasured mucous pH or viscosity or electrical conductivity.

And here's the rub: For all these methods, if pregnancy occurred then the breeder believed they had the definitive method that would work in any situation.

RETROSPECTIVE OVULATION DATE CALCULATION

What has been definitively calculated is that gestation (pregnancy) occurs 63 days (+or-2) days after ovulation. The plus or minus two days in canines is problematic from two points of view. Firstly, it's impossible to exactly pinpoint which day ovulation DID occur. Secondly, the cessation of function of the corpus luteum (the structure that maintains pregnancy) needs to be evaluated. If the corpus luteum has undergone involution and no longer produces progesterone, the placental function becomes compromised and parturition MUST occur or pups will die.

If the bitch undergoes normal parturition then she must have ovulated some 63 days prior and one can confidently state, but still with a 48 hour margin of error, which day of her cycle she did in fact ovulate. The issue here is that no two cycles are necessarily identical in any one bitch. So retrospective methods are interesting but fail to be predictors for future oestWrus cycles.

VAGINAL CYTOLOGY

This became very popular as it can be reasonably accurate to define the stages of the cycle, viz: proestrus, oestrus, diestrus and anestrus. The method requires the taking of serial samples of cells from the anterior vaginal wall to examine the cell component of the vagina and, most importantly the changes that occur through the cycle. A one off sample is not particularly informative as it is more important to note the changes in cell structure from one sample to the next.

The issue is that you do need a good quality microscope and the operator must know such things as where to take samples from (taken to far caudally, the samples are meaningless), how much normal saline is on the swab tip to take the sample, how to place the sample correctly on the slide, drying it properly is imperative, fixing the cells to the slide, staining and drying again then finally how to interpret the cells visualized especially understanding such things as the importance of foam cells when they arise.

Cytology however, is also reflecting the changes in the vagina as they occur as a result of oestrogen, for it is this hormone that mostly effects the cells of the vaginal tract.
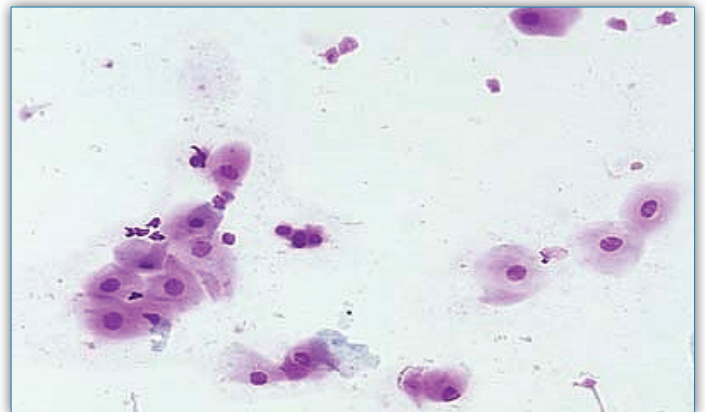
Above: Pro-Oestrus Cells. – nucleus still prominent
Opposite Page (top left): Cornification Indicates Oestrus Period.
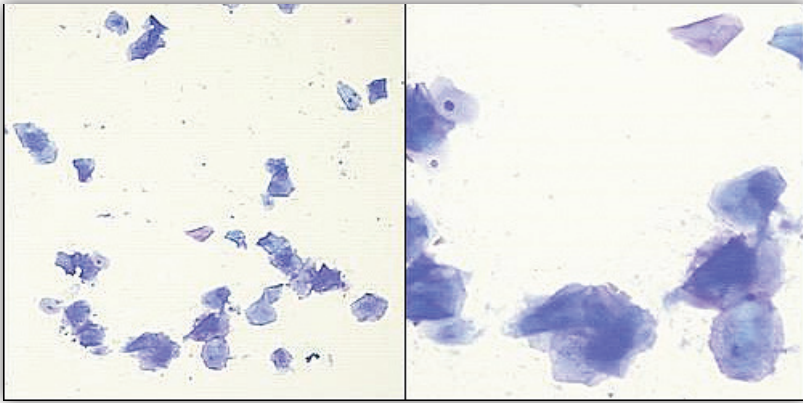
LUTENISING HORMONE ASSAY

Naturally, measuring Lutenising Hormone (LH) levels is the gold standard to determine when ovulation will occur. In the bitch this is 24 to 48 hours post the LH surge. The problem is that the hormones produced by the pituitary gland, such as LH, are more species specific – that is to say, the assay for human LH cannot be used to determine canine LH. Further, the Eliza tests that are available are highly unstable and somewhat unreliable. Finally, to assay the surge properly it would be absolutely essential to take blood daily (if not twice daily) to ensure the LH rise is not missed. This would prove extremely expensive.
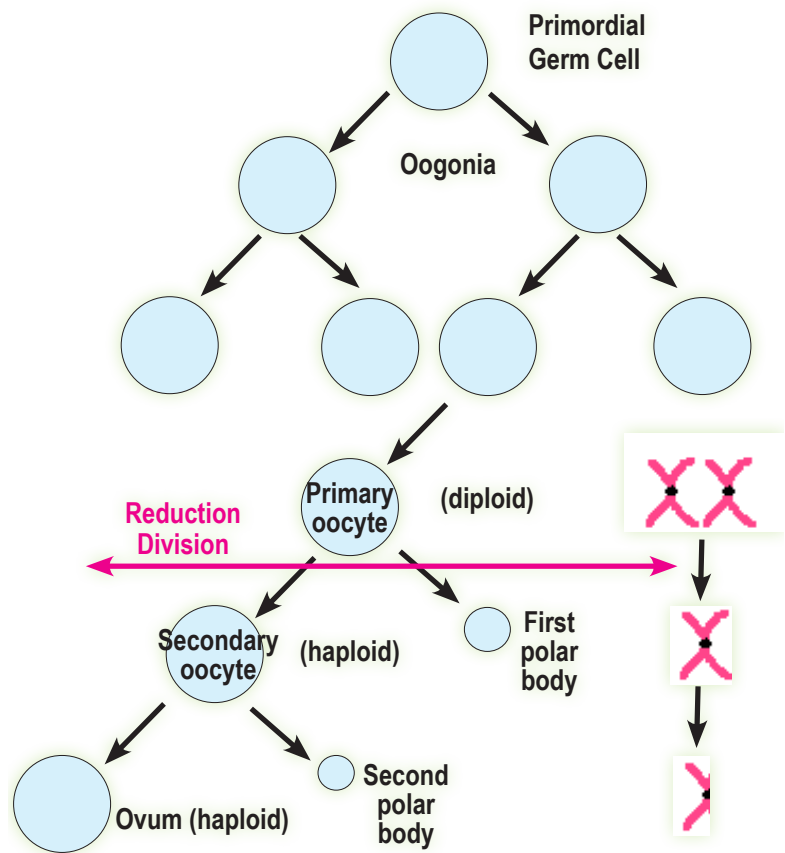
PROGESTERONE ASSAY

This has become the most common method of predicting ovulation and the fertile period in canines. Unfortunately, it is poorly understood by both breeders and many veterinarians. Commonly, people ask the question, "What level should they be at before I mate or inseminate?" This immediately assumes that all bitches rise at the same rate and that all breeds have the same ovulatory & luteal patterns, which, as we all know, is a totally erroneous conclusion.

Worse still, is the assumption that once ovulation has occurred (eggs are released from the ovary and into the ovary duct) you should immediately place semen in the tract so that fertilization can occur. Nothing is further from the truth in canines! To explain this we must take a brief look at ova (egg) development. In ever mammal the ova or egg is formed when a germinal cell forms a primary oocyte (egg). This primary oocyte must divide again to form a Secondary oocyte which is then released (ovulation) as a mature ovum (egg) ready for fertilization. **This occurs in every species except canines!**

In canines the oocyte is released BEFORE the division to the secondary oocyte occurs. Thus, the eggs that are released from the bitch's ovary are NOT ready for fertilisation.

So the added complication is that not only do we need to calculate when the bitch has released eggs BUT we also need to calculate when those eggs are going to be ready for fertilisation.

Above: Cell Division to for Oocyte(egg) - Meiosis in Females

And the big hits just keep on coming! Because the next problem arises from the fact that in the bitch progesterone begins to rise because of lutenisation of small follicles that do not produce ova. And if you believe that's the end of the problems, I have some very cheap Sydney waterfront land I can sell you but it has to be a low tide. The next issue is that bitches will ovulate between 24 to 48 hours after the progesterone rise, though we always count it as 24 hours post the rise.

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Explaining the graph to the right that depicts the bitch's hormonal pattern it is imperative that one realises that progesterone rises AS Lutenising hormone rises not after. In fact, in some bitches this occurs just prior to the LH surge.

Consequently, the day progesterone begins to rise is the most critical day and is referred to as DAY ZERO.

There are also important breed variations. Some breeds lutalise earlier and hence inseminations needs to be a little later that the average. Many breeds (Afghans, Greyhounds and most others) follow the typical pattern as the graph above. And of course other breeds start to produce progesterone some time after the LH rise so must be inseminated a little earlier.

Currently, rise in progesterone is the gold standard for detecting when eggs are released. From there, one must extrapolate as to when the eggs are ready for fertilisation.

Because fresh semen can last so long in the bitch's reproductive tract, pinpointing the exact day of the rise is not as critical. Hence, a rule of thumb for FRESH semen inseminations or natural matings is to mate two days after the bitch reaches 15 nmol/l, then 48 hours later. In some breeds it is recommended three days post reaching this figure as they lutalise early. However, as has been stated, semen can last six day plus in the bitch so if you mate at a lower rate you still could easily get a pregnancy. Similarly, the eggs will last a number of days so mating earlier can also result in a pregnancy.

Frozen semen requires exact analysis of the progesterone rise to estimate the exact timing of insemination. This is due to the short life of post-thaw frozen semen.

Some people will recommend insemination when a bitch reaches, say 50nmol/l. This of assumes that all bitches rise at the same rate!. A better method is to monitor the base line progesterone, calculate when the rise occurs then extrapolate forward to the insemination date.

At our hospital we require three parameters to determine the best date to inseminate with frozen semen:

1.   Four to six days post the rise of progesterone from baseline.
2.   Two to three days post 25nmol/l
3.   Over 50nmol/l on the day of insemination
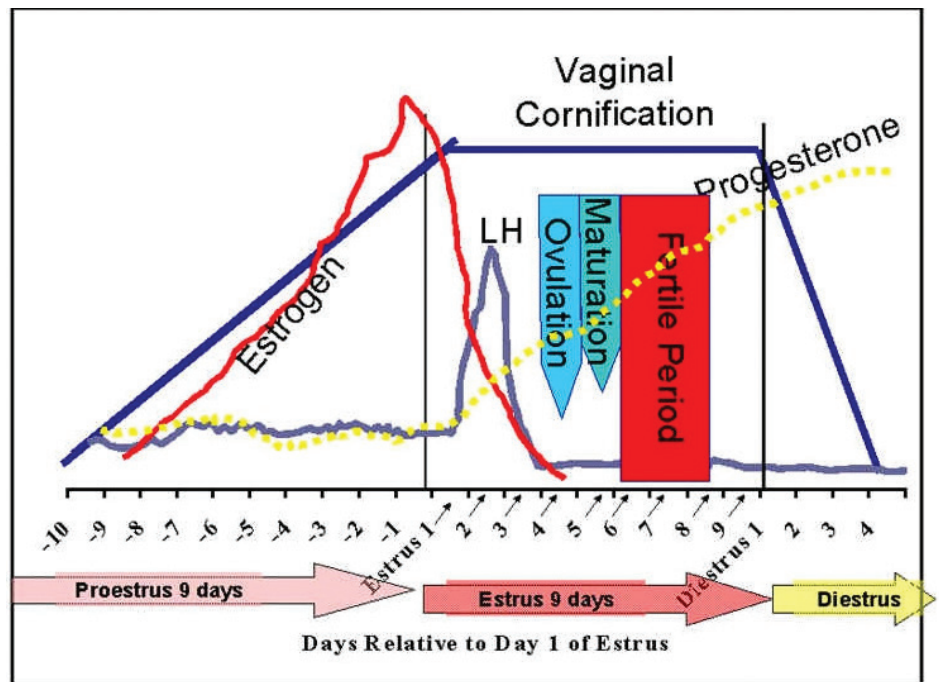
'So if you have learnt anything, let's reutirate: The most important date in calculating the fertile period in the bitch is to calculate the day of the progesterone rise off baseline.'

But even this rule is complicated by the physiology of the female canine's reproductive cycle.

Variations in any individual can be seen with some females ovulating 3 to 4 days after the start of bleeding while others are as late as 25 to 28 days post commencement of bleeding (Linde Forsberg, 2007). The only consistent parameter is that all bitches ovulate after the LH surge. This was shown by Concannon in 1975, but the problem is that ovulation will occur between 24 to 96 hours after the surge. Later, Boyd (1993) was able to demonstrate that ova are released from one ovary at a time and the whole process takes 36 to 48 hours. The oocyte must expand and mature, a process that takes 2 to 5 days and mature eggs MAY stay alive and fertilizable for 2 to 4 days. Because of these variations we know that ovulation occurs when progesterone levels are between 12 to 24 nmol/l.

THE FUTURE

So what are the possibilities of overcoming the problems created by the accumulative effect of all these variations?

*   Increasing post thaw longevity
*   Discovery of an enzyme/hormone/chemical that is only secreted when fertilizable ova are present in the oviduct
*   High powered imaging.

All these things and more are waiting to be discovered to unravel the mystery of the female canine reproductive cycle.