

## **CHECK LIST FOR FROZEN SEMEN INSEMINATION OVULATION TIMING FOR VETERINARIANS**

Thank you for your assistance with the ovulation timing for this bitch

Timing is to begin on day **one to three** of the heat cycle.

Blood needs to be collected every day (including Sundays and public holidays)

Please send all samples to the same laboratory.

Please instruct the laboratory to fax a copy of the progesterone results to Auckland Veterinary Services. Fax (09) 818 4030

On Monday                      Send the samples collected on Saturday, Sunday and Monday to the Laboratory. Ask them to process the Monday sample and hold the Saturday and Sunday samples.

On Wednesday                Send the Tuesday and Wednesday samples to the laboratory. Ask them to process the Wednesday sample but hold the Tuesday sample

On Friday                        Send the Thursday and Friday samples to the laboratory. Ask them to process the Friday sample but hold the Thursday sample.

### **IMPORTANT**

Please do not place the blood sample in the refrigerator for at least 2 hours after collection. Chilling before this time can cause a drop in progesterone level.

Please continue this until we contact you, thank you for your help

Information regarding the insemination and whelping dates will follow shortly after the insemination.

For more detailed information refer to pages 2-6

# OVULATION TIMING FOR FROZEN SEMEN INSEMINATION

It is important to remember that frozen semen, once thawed out and implanted into the uterus, has an average lifespan of only 12 hours. Thus insemination must be performed during the short 3-day fertile period of the bitch if the maximum success rates are to be obtained. AVS strongly recommends using laboratory-based tests that identify the LH peak and consequently allow us to predict the most fertile period for frozen semen insemination to be performed.

The common indicators of heat and ideal breeding time, such as flagging, vaginal swelling, vaginoscopy and vaginal smears, are all affected primarily by changes in levels of the hormone oestrogen. Unfortunately, these changes are not always accurate predictors of ovulation and can vary by more than a week when compared to the bitch's fertile period, especially considering individual interpretations.

A much more precise way of predicating ovulation and the subsequent fertile period is to measure the luteinizing hormone (LH) surge. This surge actually triggers ovulation. **Ovulation occurs 2 days after the LH surge.**

The oocytes require an additional 2 to 3 days to mature before fertilization can occur. Mature oocytes live for 48 to 72 hours. **Thus, the most fertile period for the bitch is on days 4, 5 and 6 after the LH peak.**

Many diagnostic and ancillary aids are available to assist in the timing of ovulation and the subsequent inseminations in the bitch. No single test or assay, however, is fully reliable or completely correlative with the exact stage of a bitch's oestrous. A single exam, vaginal smear or assay result provides limited information. Ovulation timing is more accurate and breeding management more successful when multiple parameters are repeatedly evaluated.

## Summary of Parameters for Timing

The following list is a summary of parameters that are useful in timing inseminations. They are ranked in order with the most accurate and reliable parameters first.

- \* The LH Assay is the most accurate of all diagnostic tests. Because luteinizing hormone is responsible for ovulation and all subsequent events, the LH assay is the most reliable for predicting the fertile period. Direct serum measurement is expensive and usually unavailable.

- \* The Progesterone Assay is an accurate measure of ovulation because the initial (Preovulatory) rise of progesterone coincides with the LH peak.
- \* Vaginoscopy is accurate, but highly subjective and requires continual practise to be useful. Additionally the degree of maximal crenulation varies between bitches.
- \* Vaginal Cytology is a simple, easy procedure that is an important aspect of a complete evaluation, but is the least accurate tool for timing inseminations when used alone. Nearly all bitches reach full cornification; however, this usually occurs before ovulation, providing limited information about the exact time of the fertile period.

### **Recommended Procedures to Maximize Accuracy of Ovulation Timing**

The following is a stepwise explanation of the timing procedures AVS recommends leading up to and after each breeding.

1. Ovulation timing should begin at the first observable signs of heat - bloody discharge, vulvar swelling, etc. Take a blood sample, in a red topped (plain) vacutainer, and send to Gribbles. At this stage, the progesterone level should be at the baseline of 0 to 1 ng/ml.

The reason for using Gribbles is that they assay progesterone using immunofluorescence and this technique is much more accurate than using ELIZA kits. They are also the laboratory we use and we have confidence in the results we receive

You will need to collect blood samples each day. Not all the samples collected will need to be analysed. Samples can be stored in the following ways.

If you have access to a centrifuge;

Collect a 5ml blood sample and centrifuge it to separate the red cells from the serum, we use 4,000 RPM for 5 minutes. Harvest the serum into a plain (red topped) vacutainer and refrigerate the sample in a normal domestic refrigerator. When serum is refrigerated the progesterone in the sample does not decay.

If you don't have access to a centrifuge;

Collect a 10ml blood sample and allow it to clot. After the clot has contracted, harvest the plasma into a plain (red topped) vacutainer and refrigerate the sample in a normal domestic refrigerator. When plasma is refrigerated the

progesterone does not decay.

On Monday send the samples collected on Saturday, Sunday and Monday to Gribbles. Ask them to process the Monday sample and hold the Saturday and Sunday samples.

On Wednesday send the Tuesday and Wednesday samples to Gribbles. Ask them to process the Wednesday sample but hold the Tuesday sample

On Friday send the Thursday and Friday samples to Gribbles. Ask them to process the Friday sample but hold the Thursday sample.

Ask Gribbles to fax a copy of the results to Auckland Veterinary Services as well as to you.

#### **Interpretation of results;**

1. Progesterone levels should rise rapidly near the time of the LH peak, from baseline to a value of 1 to 2.5 ng/ml. Following the LH peak the progesterone levels will continue to rise reaching levels well above 5ng/ml. **By definition, the day of the first rise in progesterone (equivalent to LH peak) is called Day 0 of the cycle**
2. The fertile breeding period should span days 3 to 7 post-LH peak. However, because of the reduced viability of thawed-out frozen semen, we recommend that insemination be performed on the 5th day.
3. Pregnancy diagnosis by palpation or ultrasonography may be performed as early as 28 days post-LH peak.
4. Whelping should occur 65 days post-LH peak, plus or minus one day.

**Due to importance of getting the ovulation timing exactly correct, if you have any queries please do not hesitate to call Mark Simpson at Auckland Veterinary Services 09 818 5697.**