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Ovulation Timing in the Bitch

Canine breeding management centers around ovulation timing. Ovulation timing is very important because it is critical to have viable spermatozoa in the bitch's oviducts at the time the eggs are mature and ready to be fertilized. In many bitches, the period of sexual receptivity (standing and flagging for the male) does not coincide with the bitch's most fertile period. Breeding the bitch based on sexual receptivity, when it does not coincide with the fertile period will result in non-pregnancy; or alternatively if the period of receptivity overlaps somewhat (either at the beginning or at the end) with the fertile period, conception may result but litter size will be reduced. Optimal breeding management is designed to time the breeding precisely during the period of maximal fertility. Not only does this result in maximal pregnancy rates and litter size but it also minimizes the number of breedings required, thus minimizing the use of the dog.

Ovulation timing requires the combined use of several diagnostic techniques: vaginal cytology, vaginal speculum examination, serum progesterone assay, and in some cases serum LH (luteinizing hormone) assay. Each technique will be discussed below.

Vaginal Cytology

Vaginal cytology should be begun at the onset of the bitch's cycle. It is usually best to begin within 2 – 3 days of the start of vulvar bleeding. In some bitches, the progression through proestrus occurs very quickly, with the bitch ovulating within 3 – 5 days of the onset of bleeding. If one waits until 7 or more days into the cycle, ovulation may have already been missed. Vaginal cytology is then performed every 2 - 3 days throughout the rest of the timing process. As the bitch progresses through proestrus there will be changes in the cell types visualized on the cytology slides. The vaginal epithelium becomes thickened under the influence of estrogen (the predominant hormone of proestrus). This thickening of the vaginal wall results in the vaginal slide becoming predominately superficial cells (cornified). Once the slide is approximately 30 – 50% superficial cells, serum progesterone testing should commence. Changes in the cell types may lag as many as 2 – 3 days behind hormonal changes which is why we begin checking progesterone concentrations before the cytology exam would appear to indicate that the bitch is close to ovulating.

In bitches that progress through their cycle quickly, progesterone concentrations may be started on the first day vaginal cytology is performed. It is not uncommon to obtain a progesterone concentration on the first day the bitch is examined to ensure that the bitch is actually in proestrus and not in diestrus (after the fertile period) since the vaginal cytology slide from the early proestrus bitch is very similar to a bitch in diestrus (past the fertile period). If the early portion of the cycle has been missed (occurs in bitches that cycle quickly, that are fastidious cleaners so have little to no discharge, that are heavily haired such that discharge has been missed, or in bitches housed outside or those not under close supervision) vaginal cytology may be performed for 7 – 14 before realizing the bitch is not progressing properly and a progesterone concentration obtained and prior ovulation diagnosed.

Vaginal cytology should be obtained through a speculum to avoid contamination of the swab with cells from the vestibule. The swab should be passed as far forward in the vaginal vault as possible, as the cell changes are more obvious and are more indicative of the hormonal changes farther forward in the vagina. In most bitches under 80 pounds, a 6" applicator swab is adequately long. In larger breed bitches, those over 80 pounds, use of a mare culture swab will be necessary to obtain a satisfactory vaginal slide.



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Vaginal Speculum Examination

Vaginal speculum examination should be performed each time a vaginal cytology slide is obtained. Just as the cell types change as the bitch progresses through her cycle, so does the appearance of the vaginal epithelium. In proestrus, the walls of the vagina are uniform pink in color and edematous (puffy or billowy). Once estrogen levels begin to decrease (late proestrus/early estrus) the vaginal wall begins to dehydrate and the blood supply to the vaginal wall diminishes. This results in the vaginal wall becoming pale pink and finally white in color and in wrinkling of the tissues (crenulation) as the fluid is reabsorbed. For the remainder of estrus, the vaginal wall appears white and wrinkled (crenulated). As the period of maximal fertility approaches, the degree of crenulation becomes the greatest. Once the bitch enters diestrus (past the fertile period), the vaginal wall regains its pink color. However, the color is not uniform, but rather blotchy pink and red. The mucosa is very irritable and if it is touched with an instrument or swab it will pucker up (rosette formation).

Speculum examination can be performed using otoscope speculums, pediatric proctoscopes, or rigid endoscopes. The choice will depend on available equipment and the size of the bitch being examined. In large breed bitches use of the proctoscope or endoscope will be necessary to get beyond the vestibule. Addition of air through the speculum may facilitate getting an adequate view of the vaginal wall.

Serum Progesterone Concentrations

Progesterone concentration begins to increase prior to ovulation in the bitch due to luteinization of small follicles on the ovaries. An indicator that ovulation is impending is the progesterone surge. This surge occurs when progesterone concentrations are around 2 ng/ml (or 6.2 nmol/l) or when the baseline progesterone concentration doubles. In many bitches, ovulation will occur 36 – 48 hours after the progesterone surge. Progesterone concentrations rise to between 4 and 10 ng/ml at the time of ovulation (12.6 and 31.4 nmol/l). Before ovulation, progesterone rises slowly, while after ovulation progesterone rises rapidly. A jump of at least 3 – 4 ng/ml (9.14– 12.56 nmol/l) in a 24 hour period, after progesterone concentrations reach 4 ng/ml, is confirmatory of ovulation. Some bitches may jump as much as 8 – 10 ng/ml in a 24 hour period after ovulation has occurred. Some bitches will stall between the progesterone surge and ovulation having values between 2.5 and 4.0 ng/ml (7.8 and 12.5 nmol/l) for several days.

Following ovulation it takes the eggs 2 days to mature and be ready to be fertilized. Ovulation of multiple follicles may occur over a 12 – 36 hour period. Once mature, the eggs are capable of being fertilized for 2 days. Therefore, the fertile period begins 2 days after ovulation and lasts for the next 3 – 4 days. Typically, the first day of breeding (day 4 after the LH surge) will occur when the progesterone concentration is between 12 and 15 ng/ml (37.2 and 46.8 nmol/l). It is recommended that ovulation be confirmed (rather than depending on normal progression of ovulatory events after the progesterone surge) because of the tendency for some bitches to stall between the surge and ovulation or for some bitches to fail to ovulate completely (due to split heats, inadequate hormone production from the brain, or abnormal follicular development). Progesterone testing is generally performed every 2 days, until ovulation is imminent and then daily until ovulation is confirmed.

Luteinizing Hormone Concentrations

The LH surge is the actual hormonal event that results in ovulation. The LH surge occurs 48 – 72 hours prior to ovulation and it lasts for 12 – 24 hours. Because the LH surge is so short, it requires daily blood sampling. Once the LH surge is detected, the first breeding should be planned in 4 days (for vaginal or transcervical inseminations), but ovulation should always be confirmed with progesterone testing. In many cases, the LH surge can be identified retrospectively – once progesterone reaches 2 ng/ml (6.2 nmol/l), the daily blood samples from the prior day can be tested for the LH surge. If it is negative, the sample from the day progesterone reached 2 ng/ml should be tested next. This can save on the expense



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of running daily LH tests and every other day progesterone testing. LH surge determination is especially helpful when a single insemination is to be performed (surgical AI or single transcervical insemination). If a single insemination is to be performed, the optimal day for breeding is day 5 after the LH surge (LH surge = day 0).