Responsible dog breeders are dedicated guardians of purebred dogs. Responsible breeders spend countless hours promoting their chosen breed in the show ring and at working events, attending educational seminars, studying pedigrees of potential litters, caring for their breeding stock and puppies, and mentoring new dog owners. Responsible breeders also pay for seemingly endless health screening tests (e.g., Orthopedic Foundation for Animals, Canine Eye Registry Foundation, breed-specific DNA tests) in their quest to breed healthy, sound, well-adjusted dogs. Responsible breeders are very aware of the unwanted dog problem and they carefully screen potential homes for the puppies they produce. A “not pregnant” diagnosis for a top breeding bitch is an emotional letdown as well as a financial loss to responsible breeders. Veterinarians have an obligation to their breeder-clients to determine the “fertile period” which is the best time to breed bitches. By doing this, the probability of a successful breeding outcome will be optimized.

Determination of the fertile period can be difficult because bitches usually have a long proestrus and estrus period and may be receptive for two to three weeks. The fertile period when the mature (secondary) oocytes are ready to be fertilized by viable spermatozoa is very short—usually only two to three days. The bitch ovulates primary oocytes which take approximately two days to mature to secondary oocytes. Canine oocytes remain viable for several days (four to five days after ovulation) which is unique since most mammalian oocytes only survive for a few hours. Canine spermatozoa can last up to 11 days. Because of these features, natural mating has lots of room for error whereas insemination timing with chilled or frozen semen must be more precise.

Collecting the most clinically relevant information about an estrous bitch increases the chance of determining the fertile period. The true fertile period lasts about three days—days 4, 5, and 6 of estrus. Failure to determine the fertile period often results in incorrect breeding/insemination times which contribute to reduced pregnancy rates and/or litter size. The most common cause of infertility in the bitch is inappropriately timed breedings/inseminations. Veterinarians utilize several techniques for estimating the fertile period, and thus the best time to naturally mate or artificially inseminate bitches. Serial testing for serum or plasma progesterone levels when supported by vaginal cytology and/or vaginoscopy is the most reliable method of determining a bitch's fertile period.

Progesterone is a steroid hormone produced by the corpus luteum (CL; plural corpora lutea) in the ovary. Its primary roles in female mammals are stimulation of endometrial secretion, inhibition gonadotropin releasing hormone (GnRH) release, inhibition of reproductive behavior (except in the estrous bitch where it works synergistically with declining estrogen) and the maintenance of pregnancy.

Progesterone testing is most commonly done on serum from correctly drawn, processed, transported, and tested blood samples. Depending on the test method used, plasma can also be used. Sampling is usually started in early proestrus to get a baseline value and is continued every other day and sometimes daily until after ovulation. Blood should be drawn at approximately the same time of day so that there is a 24 or 48 hour period between samples. Blood collection tubes from different manufacturers may yield differing progesterone values depending on materials and additives, including gels or physical barriers, clot activators and/or anticoagulants. Centrifuging samples before a complete clot forms may result in the presence of fibrin which may cause erroneous values. Therefore, make sure that complete clot formation has taken place prior to centrifugation of samples. Ethylenediaminetetraacetic acid (EDTA) should not be used as an anticoagulant nor should gel barrier tubes be used when processing samples by chemiluminescence (CLIA) because the progesterone levels have been shown to be either decreased or increased. Hemolyzed blood samples can give lower than normal results. Serum samples should be refrigerated if immediate testing is not done and/or frozen if being sent to a commercial laboratory. Serum progesterone concentrations can be altered by sample handling so it is recommended that veterinarians carefully read the instructions for in-house test kits or contact their
commercial laboratory for specific details concerning collection, processing, storage and shipping serum samples.

The initial rise in progesterone occurs at the time of the luteinizing hormone (LH) surge and can be used to indirectly identify the LH surge (day 0). Luteinizing hormone is a gonadotropic hormone released by the anterior lobe of the pituitary and is responsible for stimulating ovulation, formation of the corpus luteum and progesterone secretion. Progesterone continues to rise at ovulation and during the fertile period. It is recommended that an additional progesterone test be done during the fertile period to ensure there is an adequate progesterone level (functioning CL).

Testing progesterone concentrations is cheaper and easier than measuring serum LH. Serum samples can be split and one-half saved for measuring LH if a more precise determination of the LH surge is required. Luteinizing hormone levels can be measured by a rapid immunomigration test (RIM) (Witness® LH, Synbiotics, Kansas City, MO), CLIA (Immulite 1000, Siemens, Deerfield, IL), a rapid radioimmunoassay (RIA) or non-radioactive enzyme-linked immunosorbent assay (ELISA; LH-Detect, Repropharm, Nouzilly, France). The disadvantages of using LH tests are that daily blood sampling is required due to the short LH surge (12-24 hours), LH is species specific, and some laboratories are reluctant to stock the reagents due to infrequent requests for LH testing.

Progesterone testing is done at a veterinary clinic or at a commercial laboratory. Veterinarians can use in-clinic ELISA color change tests kits. Enzyme-linked immunosorbent assay involves a series of well-controlled steps designed to determine the presence or absence of specific hormones. Under more sophisticated laboratory conditions, ELISA can also determine the quantity of hormone present. They are easy to perform, inexpensive, no radio-isotopes are required (no human health/safety hazard issues) but are less accurate than RIA or CLIA as they only determine a range of progesterone (qualitative progesterone value). This makes it difficult to accurately identify the day of initial rise in progesterone or the true fertile period. Qualitative analysis may be adequate for natural mating. There are several in-clinic qualitative test kits on the market. For instance, PreMate™ (Camelot Farms, College Station, TX) and TARGET™ Canine Ovulation Timing Test Kit (BioMetallics, Princeton, NJ).

Veterinarians can also purchase machines for use in their clinics similar to the ones used at commercial laboratories so that a progesterone value in ng/ml or nmol/L is determined (quantitative progesterone value). These units can be converted with the following calculation: ng/ml x 3.18 = mmol/L. Examples of in-clinic progesterone testing machines used by veterinarians are: Immulite 1000, (Siemens)–chemiluminescent immunoassays; Mini Vadas (bioMérieux, Etoile, France)–enzyme linked fluorescent assay (ELFA); and Tosoh A1A 360 (Tosoh Biosciences, South San Francisco, CA)–fluorescence enzyme immunoassay.

Commercial laboratories use the more expensive RIA or CLIA methods of determining an absolute progesterone value. Radioimmunoassay involves the use of radioactive hormones. In the test tube, radioactive hormone competes with the same hormone from the animal’s blood that is not radioactively labeled. The amount of hormone that binds is inversely proportional to the concentration of unlabeled hormone in the animal’s blood. This technology requires specialized radioisotope-approved laboratories, expensive isotope detection equipment and the need for expensive disposal methods. Chemiluminescent immunoassay is a sensitive and economical alternative to ELISA. It does not require long incubations. The CLIA kits are designed to detect glow-based chemiluminescent reactions. The intensity of the emitted light is proportional to the amount of enzyme present and is inversely proportional to the amount of unlabeled progesterone in the sample. The concentration of progesterone in the unknown sample is quantified by comparing to a series of progesterone standards. Radioimmunoassay and CLIA are used to indirectly determine the day of ovulation and should be used for frozen semen surgical inseminations or when breeding to a subfertile male.

A recent review of the physiology of ovulation found that during anestrus progesterone values are less than 1 ng/ml. During the LH surge, progesterone values abruptly rose from <0.5 ng/ml to around 2 ng/ml (peak LH surge). Ovulation occurred approximately two days after the LH surge when progesterone values were >5 ng/ml. Noting the abrupt rise in progesterone to >5 ng/ml is a more reliable indicator of ovulation than measuring the LH surge alone. The bitch ovulates primary oocytes that
require two to three days to mature to secondary oocytes. The fertile period starts on approximately day four of estrus when progesterone levels are 6-10 ng/ml. Natural mating or artificial insemination should occur two to three days after ovulation and insemination with frozen semen three to four days after ovulation. An additional advantage of progesterone testing for breeding management is that parturition occurs 64-66 days after the LH surge and 62-63 days after ovulation.17

Summary
The most common cause of infertility in the bitch is inappropriately timed breeding. An accurate as possible determination of the LH surge (day 0), ovulation and the fertile period by performing serial progesterone tests and breeding/inseminating at the appropriate time greatly improves the chances of a “pregnant” diagnosis.

References