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MANAGING CANINE REPRODUCTION THROUGH SERUM P_4

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iVets Centre Veterinari, clinic of Kitican Veterinary Group, is a leading center in canine reproduction founded in 2014 by Daniel Casanova Reig, veterinarian and breeder with over 25 years of experience.

Their focus is mainly on the control and reproductive pathology of the canine species and has a frozen canine semen bank with more than 500 specimens of all breeds. The center collaborates very closely with about 200 breeders in the national and international territory and performs annually about 1,500 progesterone tests (P_4), hundreds of artificial insemination procedures in all its variants and about 200 caesarean sections, a procedure that results in the birth of more than 1,000 puppies in this center.

In the process of confirmation and control of gestation, we perform ultrasound controls through which we can diagnose fetal pathologies at an early age and endocrinological controls, especially of P_4, for the rare cases of primary luteal insufficiency in the female dog, a pathology characterized by a low production of progesterone in the corpus luteum during gestation. Finally, thanks to the combination of several factors, our pregnancy rate with frozen semen in 2022 was 98%.

During the last decades, the pet business has undergone a very significant growth, reaching a figure of 13% in the last three years. The association of animal food manufacturers and veterinary industry estimated a census in 2021 of 29 million pets, including dogs, cats and other animals. In comparison, this represents an increase of 2.6 million dogs and 2.1 million cats compared to 2019. In parallel, we have been witnessing a drastic change in the method of reproductive control in the female dog, especially in terms of identifying the optimal days for mating or insemination of future mothers and also in the scheduling of cesarean sections, if necessary. The use of quantitative assessment of P_4 has displaced other methods, such as vaginal cytology, estrus day counting and estrous behavior of the dog, as

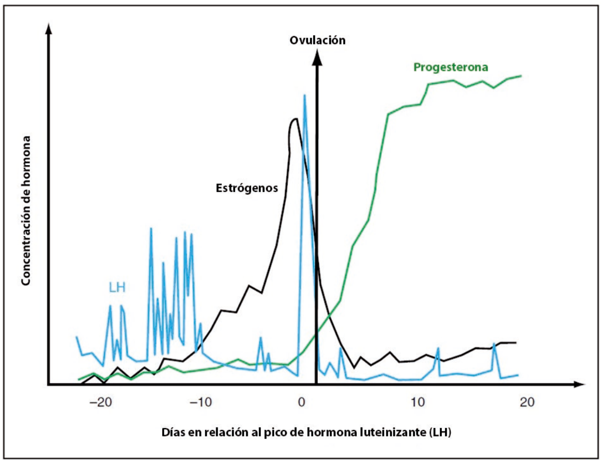

Figure 1: Preovulatory hormonal changes: estrogen, LH and progesterone concentrations in the dog

it is a more accurate method and does not entail an excessive cost for the breeder. In addition, the massive introduction of more complex reproductive techniques, such as intrauterine inseminations by endoscopy or laparotomy with chilled or frozen semen, make it imperative to detect the day of ovulation by serum P_4. Although day 0 is generally considered to be the moment when the peak of luteinizing hormone (LH) occurs, the difficulty involved in detecting this condition, as well as the high cost involved, it is currently more practical and cost-efficient to use P_4 for the detection of the day of ovulation in the dog.

The reproductive physiology of the dog is, to say the least, peculiar. Her non-seasonal monosex pattern with an obligatory anestrus probably reflects the consequences of the domestication of a species with a highly organized social and reproductive structure that was initially designed for seasonal reproduction. In the vast majority of domestic species the interval between ovulations is approximately 21 days and the estrous cycle usually consists of the phases: estrus, luteal phase (the period of preparation for gestation which is usually divided into metaestrus and diestrus) and proestrus (the period of preparation for the return to estrus). During gestation the luteal phase is extended, and in some species there are periods of sexual inactivity (anestrus) which are usually controlled by the photoperiod; that is, in relation to the seasons. The cycles of the female dog differ from this general pattern in that the periods of proestrus and estrus are extended and accompanied by an obligatory luteal phase regardless of whether gestation occurs. These periods are followed by anestrus, which is not seasonally regulated. Thus, the average cycle length is approximately 7 months, although this can vary significantly among individuals.

The fundamental endocrinological events in the female dogs are similar to those of all other species since all of them have an LH peak approximately two days prior to ovulation. However, the fundamental difference in this species is that at the time of ovulation the oocytes are immature and cannot be fertilized immediately, and fertilization can only occur once the first polar body has been extruded and the first meiotic division is completed to form the secondary oocyte. This maturation occurs 48 to 60 hours post-ovulation, and the oocytes remain viable or fertile for several days. Compared to other domestic species, this delay in the availability of oocytes for fertilization, together with their long survival, has a significant impact when scheduling artificial inseminations, especially when considering the use of chilled or frozen semen. This is the fundamental paradigm we face in the reproductive management of the domestic dog and we must rely on serial P_4 results to decide the optimal timing of insemination.

Progesterone was first isolated in 1934 from the ovaries of female pigs by Allen and Wintersteiner. Its systematic name is pregn-4-en-3,20-dione and its molecular formula is C21H3002. It is a C-21 steroid hormone involved in the menstrual cycle of all mammals and is synthesized from cholesterol in ovarian tissues, testicular tissues, fetoplacental units and adrenal glands. Within the mitochondria of these tissues cholesterol is transformed into pregnenolone by a multienzyme complex responsible for limiting the rate of gonadal stereidogenesis. Pregnenolone is converted to progesterone by the action of a microsomal enzyme complex, and is either secreted by luteal cells or used as a substrate for androgen and estrogen production in the testes and ovarian follicles, respectively.

At the time of ovulation, the vast majority of dogs have serum P_4 values between 4 and 10 ng/ml, and, in addition, there is an abrupt increase of at least 2 ng/ml in 24 hours.
These parameters allow us to identify with a certain degree of certainty the moment when ovulation occurs, which can also be confirmed by ovarian ultrasound and, with less precision, by vaginal cytology. In this way, and taking into account the above, we can calculate the fertile days during which we should perform mating or artificial inseminations. Unlike other species, canine progesterone is produced exclusively in the corpus luteum, which is located in the ovaries, during the luteal phase (with or without gestation). The gestational luteal phase lasts approximately 63 days from ovulation and 66 days in the non-gestational luteal phase.

During the second half of the luteal phase, P_4 levels begin to decline sharply, and an abrupt decline occurs at the end of gestation. In other species this decline in P_4 occurs as a result of cortisol production by the fetuses, resulting in increased levels of enzymes that convert P_4 to estradiol and 17-hydroxyprogesterone. It should be mentioned that there are significant differences in opinion among veterinary groups regarding the levels of P_4 necessary to maintain gestation, and there does not seem to be a general consensus on this matter. Thus, protocols for action in the face of certain serum P_4 levels during gestation vary significantly among experts. Some professionals prefer, not without risk, to supplement with synthetic progesterone at certain levels while others are more tolerant of low levels of P_4 during gestation. Certainly, there are differences in criteria regarding the minimum acceptable levels of P_4 during gestation in the domestic dog and it is not the purpose of this monograph to delve into the matter.

Clinical cases

As a first clinical case example we present Nathalie, a 2-year-old primiparous German Shepherd female dog. The owners wish to perform a natural mating in Germany and want to know approximately when they should make the trip. On the eighth day of estrus the serum P_4 is 1.3 ng/ml and it is decided to repeat in 48 hours. The result obtained is 2.8 ng/ml, a value that coincides with the pre-ovulatory LH peak. The owners are told that if everything is normal, Nathalie should ovulate in 48 hours and then they can travel to Germany for mating. In fact, two days later the P_4 value was 8.3 ng/ml. This day is established as the ovulation day and two matings are performed one day apart. Twenty-one days after ovulation, an ultrasonographic examination is performed and gestation of 7-8 embryonic vesicles is confirmed, and a P_4 control is performed, with a result of 19.5 ng/ml. The gestation developed without apparent incidents and on the 61st day Nathalie came to the office for an end of gestation x-ray and a P_4 test. On this day the P_4 value is 3.1 ng/ml and the owner is informed that the calving should occur, and it does, in approximately 48 hours.

On the other hand, Puma, a 5-year-old Rottweiler female dog who has had 3 litters in the past with a history of resorptions and mummifications in the last gestation, presents for consultation. On the 10th day of estrus the P_4 is at 4.1 ng/ml and imminent ovulation is suspected. In this case the male is also abroad, but the owners do not want to make a long trip and opt to receive refrigerated semen from the male. P_4 is repeated the next day to confirm ovulation and the P_4 value is 4.6 ng/ml. It is explained to the owner that ovulation has not yet occurred as there has not been an increase of > 2 ng/ml in 24h. Thus, P_4 is repeated during the following days and when ovulatory failure is suspected, 4 days later the P_4 is found to be 9.2 ng/ml. Refrigerated semen is sent and inseminated by endoscopy (TCI) 72 hours after ovulation.

Twenty-two days after ovulation, an ultrasound was performed to evaluate gestation and 5 vesicles without embryonic content were observed, in addition to two structures compatible with reabsorptions. P_4 was evaluated and the value obtained was 4.2 ng/ml, obviously a value much lower than expected at this stage of gestation. The owner suspects primary luteal failure and requests that the dog should be supplemented with synthetic progesterone to "maintain" the gestation. At the same time, several serological tests are sent to the laboratory and a positive IgG and IgM result for Toxoplasma gondii is obtained. Thus, fetal resorption caused by Toxoplasmosis and not by primary luteal failure is diagnosed. A 28-day treatment with clindamycin is performed and in the next heat, reproductive control with P_4 and ultrasonographic study is performed. Ovulation was detected with a P_4 level of 7.6 ng/ml and 11.2 ng/ml 24 hours later. Two inseminations with refrigerated semen were performed on the second and fourth day post-ovulation and gestation was confirmed at 22 days. The P_4 at this time is 16.9 ng/ml. At the express request of the owner, P_4 levels were checked every 5-7 days and remained above 14 ng/ml. Despite the owner's insistence, it was decided not to supplement with synthetic P_4 and on day 62 the P_4 was at 1.2 ng/ml, and at this time a programmed cesarean section was performed, from which 6 vital puppies were born.

Conclusions

We can obtain very reliable serum P_4 values in a short period of time and in a very simple way. This allows us to act quickly to detect ovulation and plan mating or artificial inseminations well in advance, as well as in situations of possible reproductive pathologies and for the immediate scheduling of cesarean sections. Likewise, in cases where P_4 supplementation is indicated, we can perform serial controls to evaluate the response to treatment.

In conclusion, for the reproductive control of the female dog measuring P_4 allows us to:

- Identify the stage of the estrous cycle in which the dog is (proestrus, estrus, oestrus, diestrous and anestrus).

- Establish with a high level of reliability the day of ovulation. P_4 values between 5 and 10 ng/ml, although this may vary between individuals.

- Monitor the development of pregnancy.

- Monitor the response to treatment with synthetic P_4 in cases of luteal failure.

- Schedule cesarean sections when necessary and safely detect the abrupt drop in P_4 in the last days of gestation.

Immunological test analyzers

Trademarks

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