

RELIABILITY AND USEFULNESS OF RELAXIN ASSAY KIT FOR PREGNANCY DIAGNOSIS IN FEMALE DOG

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ABSTRACT

Pregnancy diagnosis was done in 16 female dogs using relaxin assay kit. Out of these, breeding was done in nine dogs based upon the indication of luteinizing hormone (LH) surge and all were diagnosed positive for pregnancy using relaxin assay kit with 66.7% pregnancies diagnosed positive on day 24 post-LH surge. The remaining seven dogs were bred based upon predetermined dates and six out of seven dogs were diagnosed positive using the assay kit, with 66.7% diagnosed positive on days 27-28 post-breeding. In brief, relaxin test is a reliable and a quick indicator for pregnancy in female dogs, but it fails to confirm pregnancy any earlier than abdominal ultrasonography.

Keywords: Bitch, Canine, Pregnancy, Relaxin, Ultrasonography

Serologic testing for pregnancy in canines is complicated because of their endocrine physiology. The estimation of serum progesterone for pregnancy diagnosis is not useful as the levels are similar in pregnant and non-pregnant dogs. Placental gonadotrophins are secreted in human (Human chorionic gonadotrophin) and equine (Equine chorionic gonadotrophin), but not in dogs. The hormone relaxin, a 6-KD polypeptide, primarily produced by ovaries and placenta of pregnant bitches was identified as a specific marker for pregnancy and is not secreted in pseudopregnant bitches (Klonisch *et al.*, 1999 and Kutzler *et al.*, 2003). Also, a sudden drop in serum relaxin is a useful indicator for spontaneous abortion (Luvoni and Beccaglia, 2006). The present paper describes the use of a commercial relaxin assay kit for pregnancy diagnosis in female dogs.

Sixteen bitches of different breeds brought for pregnancy diagnosis to the Canine Fertility Clinic, Chennai were included in the present study. In nine bitches (Group I), breeding was done based upon progesterone estimations where progesterone concentrations of 1-3, 4-10 and 11-22 ng/ml were indicative of Luteinizing hormone (LH) surge,

ovulation and fertilization period, respectively (Sridevi and Veerapandian, 2011). Bitches were bred twice during the fertile period. The remaining seven (Group II) bitches were bred based on predetermined dates, the most common being days 9, 11 and 13. Serum samples were obtained between day 20-28 after LH surge or after breeding from Group I and II dogs, respectively. The serum samples were tested for relaxin using a commercial relaxin assay kit based on Rapid Immuno-Migration (RIM) Technology, using the combination of anti relaxin antibodies to quickly identify the relaxin hormone in canine serum or plasma samples. All the dogs were also subjected to ultrasonography at day 30 post-breeding to diagnose pregnancy and to check the efficacy of assay.

All the bitches in group-I were diagnosed positive for pregnancy using the relaxin assay kit with 66.7% pregnancies diagnosed positive on day 24 post-LH surge. The pregnancies were also confirmed using ultrasonography indicating that there were no false positives with the assay. In another study, the day that pregnancy was detected using relaxin assay ranged between day 19-28 post-LH surge (Buff *et al.*, 2001). In group II, six out of seven bitches were diagnosed positive using the assay with 66.7% diagnosed positive

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on days 27-28 post breeding. Out of two bitches that were declared negative on day 26 post breeding using the relaxin kit, one was diagnosed positive using ultrasonography on day 30 and the bitch delivered two live puppies indicating that false negative results may occur when pregnant bitches carried small litters and/or one or more of the puppies were non-viable. Hence, all negative cases should be retested after one week. Similarly, false negatives also occur when tests are performed in relation to the day of mating and not to the day of LH surge or ovulation. This is because of the fact that canine sperms are capable of surviving for 6-7 days in the uterus (Concannon *et al.*, 1983), thus making the early detection of pregnancy post-breeding highly variable.

In brief, relaxin test is a reliable and a quick indicator for pregnancy in female dogs, but it fails to confirm pregnancy any earlier than abdominal ultrasonography, nor does it provide information about fetal viability or litter size. Hence, abdominal ultrasonography is a preferred method for diagnosing pregnancy in bitches. The relaxin assay may be useful when the access to ultrasonography is not possible or the dogs are not handled regularly.

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