

## Relaxin as a diagnostic tool for pregnancy in the coyote (*Canis latrans*)

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### Abstract

The diagnosis of pregnancy in the domestic dog (*Canis familiaris*) often employs specialized equipment, experienced staff, and the cooperation of the bitch. These procedures can be challenging when the subject is a wild canid, particularly in a field setting. In addition, reproductive hormone assays are unreliable as a diagnostic tool because the estrous profiles of pregnant and pseudopregnant canines are similar. However, research has demonstrated that the hormone relaxin can be detected in maternal blood after embryonic implantation, but remains negligible in non-pregnant females. We investigated the use of relaxin as a diagnostic marker of pregnancy in the coyote (*C. latrans*). A commercially available canine relaxin enzyme immunoassay (ReproCHEK<sup>TM</sup>) was used to test plasma collected from 124 female coyotes over four consecutive breeding seasons. Mating activities of the captive females were observed; then peripheral blood samples were collected at intervals throughout pregnancy, as well as after parturition. Results demonstrated that relaxin could be detected in the plasma of pregnant coyotes after 28 days of gestation, and in some cases as early as 23 days, while non-pregnant females and male coyotes consistently tested negative. Relaxin also remained detectable in the plasma of the majority of females tested 10–12 weeks after parturition. This qualitative assay for relaxin proved to be a reliable diagnostic tool for pregnancy in the coyote. In addition, blood sampling was relatively easy, could be accomplished with minimal handling, and did not require sedation or anesthesia.

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## 1. Introduction

The coyote (*Canis latrans*) is a medium-sized wild canid, native to North America and closely related to the gray wolf (*C. lupus*), red wolf (*C. rufus*), and domestic dog (*C. familiaris*) (Roy et al., 1994). Coyotes are considered seasonally monestrous (Hamlett, 1938; Gier, 1968) and socially monogamous (Andelt, 1985; Bekoff and Wells, 1986; Gese, 2001). While there is some regional variation in the actual breeding season, coyotes generally mate in mid- to late-winter, have a gestation period of 60–63 days, and deliver an average litter of three to seven pups in the spring (Hamlett, 1938; Gier, 1968; Knowlton, 1972; Gese et al., 1989). Typically, only the dominant male and female within a coyote social group produce a litter (Gese et al., 1989, 1996), although subordinate associates will help defend the pups and territory (Andelt, 1985; Bekoff and Wells, 1986; Gese, 2001).

The reproductive hormone profile of the coyote's estrous cycle has been studied (Stellflug et al., 1981; Hodges, 1990) and appears to share certain features with the patterns described for the wolf (Seal et al., 1979; Kreeger et al., 1991; Walker et al., 2002) and domestic dog (reviewed in: Concannon et al., 1989, 2001). Like the wolf and dog, differentiating a pregnant coyote from a non-pregnant female can be difficult if based on serological assessment alone. Pregnant and pseudopregnant coyotes have similar patterns of serum progesterone secretion, and the absolute concentration levels vary widely among individuals (Carlson, 2006). Also, while mid-gestation prolactin levels in coyotes rise significantly above those observed in non-gravid diestrous females, the absolute values still overlap (Carlson, 2006). Thus, single blood sampling for progesterone or prolactin seems unreliable as a method of determining reproductive status in coyotes. The hormone relaxin, however, has not yet been explored in this species.

Relaxin is a polypeptide shown to affect the reproductive tissues of mammals, most commonly "cervical extensibility and uterine contractibility" (Sherwood, 1994). The source of relaxin synthesis varies among species, but the predominant sites are the corpus luteum, placenta, and uterus (Sherwood, 1994). Depending on the species, detection of relaxin in peripheral blood is not always restricted to pregnant females; however in the domestic dog it has been established as a pregnancy-specific hormone (Steinetz et al., 1987, 1989). The site of synthesis in the bitch has been elucidated (Tsutsui and Stewart, 1991; Klonisch et al., 1999) and primarily ascribed to the placenta, although the hormone can also be traced in the ovary and uterus. These latter tissues may be areas influenced by the paracrine deposition of relaxin. A clinical study of domestic dogs using the commercially available canine relaxin enzyme-linked immunoassay (ELISA) ReproCHEK™ (Synbiotics Corporation, San Diego, CA, USA) reported detection of the hormone in maternal peripheral blood as early as 25 days after ovulation (Buff et al., 2001).

In support of a longitudinal investigation in the reproductive biology of the coyote (Carlson, 2006), a diagnostic tool was needed to easily distinguish between pregnant and pseudopregnant females, while minimizing research induced disturbances. Although there are behavioral and physiological differences between the coyote and domestic dog, there are many common reproductive features (Gier, 1968; Silver and Silver, 1969; Kennelly and Johns, 1976; Kennelly, 1978). Therefore, we tested the use of relaxin as a serological marker of pregnancy in the coyotes with the hypothesis it would be as successful in a wild canid as it has been in its domestic congener.

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