Canine Relaxin (C-RLN)

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a. Overview of Canine Relaxin

Relaxin (RLN)

Relaxin is a peptide hormone that facilitates childbirth. It is produced by the ovarian corpus luteum, uterus and placenta of female animals, its physiological function is based on the action of oestrogen.

A few days before delivery, it can loosen the tightly closed pelvic ligaments, relax the uterine muscles and increase the weight of the uterus, so the birth canal is ready to facilitate the delivery of the foetus.

a. Specific Markers for Pregnancy Diagnosis

By acting through its receptors (RXFP1, RXFP2), relaxin (RLN) exerts species-specific effects during pregnancy; possible luteotropic effects through stimulation of prolactin (PRL) release have been suggested. In the domestic dog (Canis lupus familiaris) serum PRL increases in pregnant bitches shortly after RLN appears in the circulation, and a possible functional relationship between the RLN and the PRL systems in regulating progesterone secretion has been implied. Therefore, here (Study 1) the luteal expression and localisation of the RLN system was investigated by immunohistochemistry using custom-made antibodies and semi-quantitative PCR, at selected time points during gestation: pre-implantation (d. 8–12), post-implantation (d. 18–25), mid-gestation (d. 35–40) and at normal and antigestagen-induced luteolysis. Further, (Study 2) hypophyseal expression of the RLN system and its spatial association with PRL was assessed. Luteal expression of RLN, but not of its receptors, was time-dependent; it increased significantly following implantation towards mid-gestation and decreased at prepartum. Antigestagen treatment resulted in downregulation of RLN and RXFP2. Whereas RLN was localised in steroidogenic cells, RXFP1 and RXFP2 also stained strongly in macrophages and vascular endothelial cells. The RLN system was detected in the canine adenohypophysis and was co-localised with PRL in hypophyseal lactotrophs. The intraluteal RLN seems to be involved in regulating the canine corpus luteum (CL) in a time-dependent manner. The presence of RLN family members in the adenohypophysis implies their possible involvement in regulating the availability of PRL and other pituitary hormones.

Luteal and hypophyseal expression of the canine relaxin (RLN) system during pregnancy implications for luteotropic function
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0191374

b. Pregnancy in Dogs

Week 1 (0-7 days)	Week 2 (8-14 days)	Week 3 (15-21 days)	Week 4 (22-28 days)
The female dog will not show signs of pregnancy at this stage. The eggs are fertilised after ovulation and mating.	The fertilised eggs will successfully reach the uterus and colonise, and the female will show mild behavioural changes.	The fertilised egg will embed itself in the lining of the uterus and continue to develop throughout pregnancy. Female dogs will show the development of mammary tissue as the first physical sign of pregnancy.	22 days of gestation. At this stage, the foetus will develop facial features and the spinal cord will develop. The relaxin level of female dogs gradually increases. The nipples will also become larger, and clear and thin vaginal secretions will appear. Appetite will also increase. Pregnancy test strips can be taken at 25-30 days.

b. Pregnancy in Dogs

Week 5 (29-35 days)	Week 6 (36-42 days)	Week 7 (43-49 days)	Week 8 (49-57 days)
The amniotic fluid in the uterus will increase, various organs will gradually form, abdominal pressure will increase and vomiting will occur. On the 40th day of gestation, the female dog's weight increases by 20%-50%.	The abdominal distension of the female dog will be obvious. Due to the increase in blood supply, the nipples will change and the female dog's appetite will further increase.	Puppies are almost fully developed. The female dog's abdomen will harden and enlarge, and she will also produce nutritious colostrum or 'breast milk' to prepare for delivery.	Foetal activity can be seen on the 50th day of pregnancy.

Week 9 (58-63 days)	Week 10 (63-69 days)
By the 58th day, the foetus is fully grown. At 55-60 days, the puppies are about to give birth and the progesterone (P) level drops. Dog owners must remain highly vigilant. The female dog will become quiet and withdrawn. Body temperature will drop around 61-62 days. 12-24 hours before the foetus is born, the body temperature will further drop to 36.1°C.	Extended pregnancy. Consult a veterinarian to check whether the mother dog and foetus are healthy.

b. Pregnancy in Dogs

1. Dogs are seasonal single-oestrus animals, and they go into oestrus once every year in spring and autumn. The time from the beginning of blood discharge from the female dog's vagina to the time she accepts mating is proestrus, which lasts about 7-13 days. The time when a female dog is receptive to mating is the oestrus period, which lasts about 3-21 days, with an average of about 9.
2. The gestation period of female dogs is generally 57-65 days and most dogs begin to give birth naturally around 60 days.
3. The pregnancy period of a female dog is divided by the last mating day. It can usually be detected on the 25th-30th day of early pregnancy.

c. Diagnostic Methods for Early Pregnancy in Dogs

By Touch

3-4 weeks of pregnancy by palpating the foetus's hard amniotic fluid sac in the uterine horns. Beaded feel, diameter 1-2cm. In the third trimester (starting at 6 weeks), individual foetuses can be palpated and foetal movements detected. After the peak of luteinising hormone appears, the foetal sac can be palpated on 22-23 days.

The foetal sac is relatively flat, difficult to palpate and has low accuracy.

c. Diagnostic Methods for Early Pregnancy in Dogs

Abdominal Ultrasound

Abdominal ultrasound is an effective method for pregnancy diagnosis in dogs. Evidence of foetal life - through the foetal heartbeat and movement - can also be detected from a Doppler ultrasound around 21 days into pregnancy. At 5 weeks into pregnancy, the foetus can be easily detected. The foetal heart can be seen and the heart rate can be calculated. Foetal number can also be assessed.

c. Diagnostic Methods for Early Pregnancy in Dogs

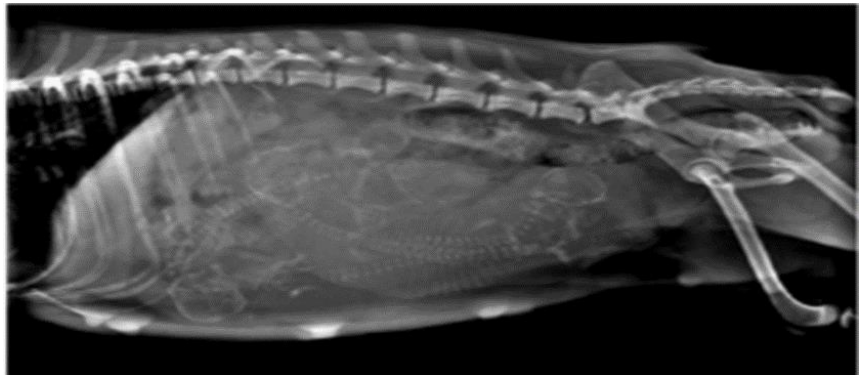
Hormone Diagnosis

Between the 25th-30th day of pregnancy, the level of relaxin in the blood of female dogs increases. Fluorescence immunochromatography or colloidal gold test is used to diagnose the pregnancy (early pregnancy) by checking the relaxin level.

c. Diagnostic Methods for Early Pregnancy in Dogs

Abdominal X-Ray Diagnosis

Pregnancy can be confirmed by an abdominal X-ray, taken after 35 days of pregnancy when organogenesis is complete. Foetal skeletal ossification can become apparent around day 40 of pregnancy. Radiographic examination of the entire abdomen can count the number of foetuses. These should be avoided if other methods of pregnancy assessment are available.



d. Clinical Application of Canine Relaxin

Relaxin is a polypeptide hormone synthesised and secreted by the corpus luteum and placenta during pregnancy. It gradually increases with the growth of pregnancy and decreases rapidly after delivery. As a kind of early pregnancy hormone in animals, relaxin is widely used for the auxiliary diagnosis of early pregnancy in animals. The concentration of relaxin in the blood increases rapidly.

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Canine Relaxin (C-RLN) Rapid Quantitative Test

Woodley have developed a rapid, accurate, reliable and highly sensitive detection method for C-RLN in canines.

The InSight V-IA Canine Relaxin (C-RLN) Rapid Quantitative Test is a fluorescence immunoassay used with the InSight V-IA Veterinary Immunoassay Analyser for the quantitative determination of C-RLN concentration in canine serum or plasma.

The test is used to diagnose early pregnancy in dogs.

It can be stored at room temperature.

Thank You

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