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Comparative Study for using Relaxin, Ultrasonography, and Abdominal Palpation for Pregnancy Diagnosis in Cats.

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ABSTRACT

This study aimed to compare different methods for pregnancy diagnosis in cats. The study was conducted in two private veterinary clinics in Al-Hilla city. The study included 11 queens, with an average of 12.8 months, who were brought to the clinic for a pregnancy diagnosis. Pregnancy diagnosis was carried out by abdominal palpation, estimation of relaxin, and ultrasonography. Pregnancy diagnosis was carried out by estimation of Relaxin rapid test. An ultrasound device with a microconvex probe (3.5–5 MHz) was used for pregnancy diagnosis. The results illustrated that 11 cases were detected as pregnant by abdominal palpation, while 9 cases were reported as pregnant when detected by ultrasonography and relaxation tests. The percentage of pregnancy rates in this study was 81%. The results showed that the efficiency of the relaxin kit was 100% in conformity with ultrasonography for positive and negative results. We conclude that the use of the Relaxin rapid test for pregnancy diagnosis in cats was accurate when made after 25 days of gestation with confirmation with ultrasonography.

Keywords: Queen, Pregnancy, Ultrasonography, Relaxin.

Introduction

Cat breeding is a popular hobby, with a substantial industry of skilled breeders that produce pedigree cats for both the show and pet markets (Levy *et al*., 2010) Queens can have many pregnancies in a single reproductive season, which makes them one of the most productive domestic species (Griffin, 2001). The estrous cycle of the female feline (queen) is distinctive within the realm of domesticated species and encompasses five distinct phases, namely proestrus, estrus, interestrus, diestrus, and anestrus. Queens exhibit a wide spectrum of individual diversity in cycle length. Female felines exhibit seasonal polyestrus and possess a high level of fertility. Both copulatory and non-copulatory stimulation have the potential to trigger ovulation (Griffin, 2001). Ovulation takes place during a time frame of 24 to 48 hours following sexual intercourse. The ova undergo fertilization within the oviduct and thereafter migrate to the uterine horns as morulae within 5-6 days following coitus. Following the process of implantation, which typically takes place approximately 13-14 days after ovulation, the pregnancy proceeds to progress with a very stable sequence of chronological occurrences. The structure of the placenta is endotheliochorial, whereas its shape is zonary. Parturition typically takes place within a timeframe of 63 to 67 days (Zambelli and Prati, 2006). Queens secrete estradiol-17², luteinizing hormone, and progesterone, which are the three main hormones of pregnancy, to different degrees during the gestation period (Kustritz, 2006). The placenta is responsible for the production of relaxin during pregnancy, which is believed to have a role in its maintenance by suppressing uterine activity. During the final trimester of pregnancy, prolactin is classified as an autotrophic hormone (Taverne and Noakes, 2018). Many techniques can be used to diagnose pregnancy in cats, such as hormone level assessment, belly palpation, radiography, and ultrasonography (Monteiro *et al*., 2011). This study aims to Evaluate the efficacy of the Relaxin rapid test for the first time in Iraq for pregnancy diagnosis and compare abdominal palpation, Ultrasonography, and Relaxin rapid test for pregnancy diagnosis in cats.

Materials and Methods

The study was conducted in two private veterinary clinics in Al-Hilla city. The study included (11) queens from different breeds (Persian, Chinchilla, Himalaya, Sherazi and Scottish) and ages (7-24) months with an average of 12.8 months brought to the clinic for diagnosis of the pregnancy. Pregnancy diagnosis was carried out by abdominal palpation, estimation of relaxin, and ultrasonography.

1- Abdominal palpation

Palpation through the abdominal wall was made by elevating the forequarter, and two hands crossed the abdominal wall to detect the amniotic vesicles.

2- Relaxin

Pregnancy diagnosis was carried out by estimation of Relaxin rapid test (MEGACOR, Austria). The blood sample was collected from the cephalic vein, and serum was obtained after centrifugation for five minutes at 6,000 rpm and used for relaxin estimation.

a) Test principle of relaxin detection

The FASTest® RELAXIN was based on an immunochromatographic "sandwich principle". The Relaxin molecules of the sample were bound to monoclonal mobile antibodies, which were bound to gold particles. Migrating ("lateral flow", LF) along the nitrocellulose membrane, these antigen-antibody complexes were bound by immobilized highly specific monoclonal antibodies against Relaxin, producing a pink-purple TEST line (B). A correct test procedure was indicated by a second, pink-purple CONTROL line (C).

b) Test procedure

1. The test cassette was removed from its foil pouch shortly before use and placed on a flat surface.

2. Thereafter, two drops (ca. 80–100 µl) of the sample were added into sample window A by using the disposable plastic pipette (Figure 1).

3. The buffer dropper bottle A held vertically and expressed two drops of buffer diluent (ca. 80–100 µl) into the sample window A (Figure 2).

4. One additional drop of buffer diluent could be added into sample window A if the LF does not start within 1 minute after adding the buffer diluent.

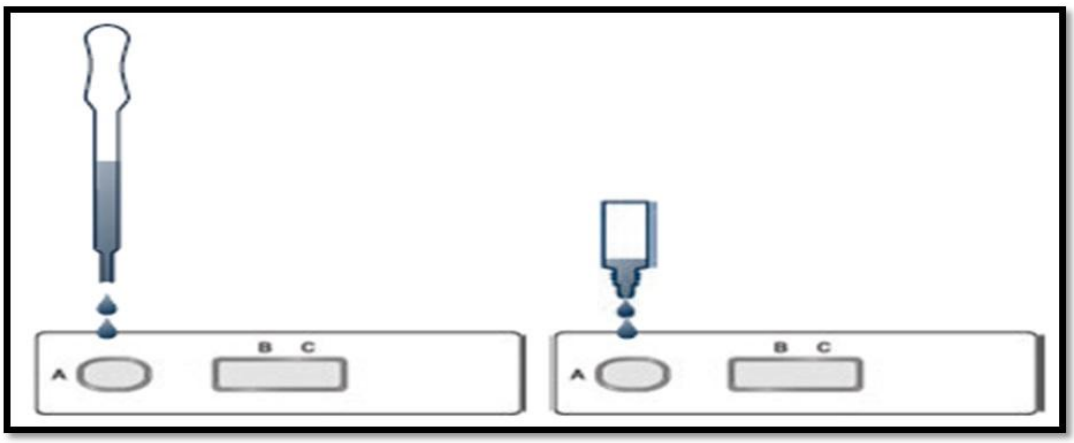

Figure 1: Procedure of relaxin kit.

Ten to thirty minutes after the addition of the two drops of buffer diluent into the sample window A, the test result was read.

c) Positive test result (Figure 2)

A pink-purple TEST line of any intensity (varied from very weak to intensely intensive) and a pink-purple CONTROL line appeared.

d) Negative test result (Figure 2)

When only a pink-purple CONTROL line appeared irrespective of its intensity, it means that the test was performed correctly.

e) Invalid test result

When no control line is visible, which meaning test cassette should be repeated.

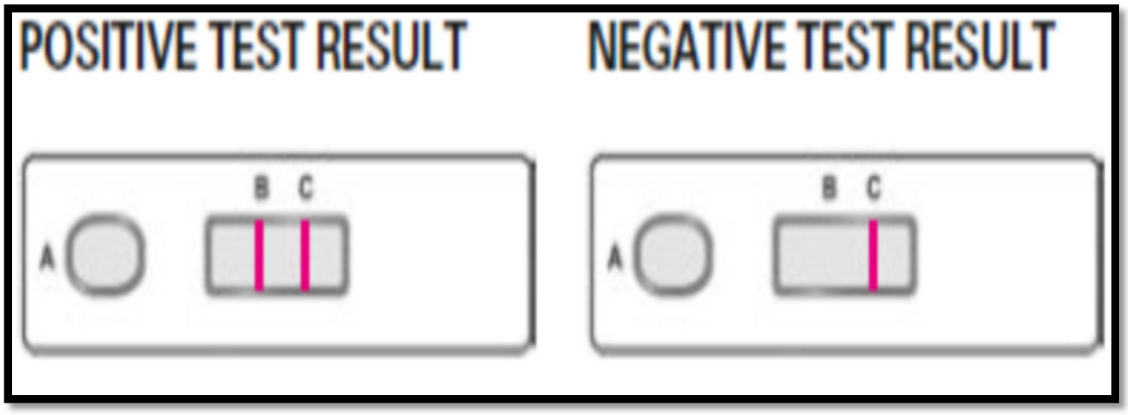

Figure 2: The positive and negative results of the relaxin kit.

3- Ultrasonography

An ultrasound device (Chison Ecovet 3, China) with a micro convex probe (3.5-5) MHz was used for pregnancy diagnosis, and the examination area was prepared for the examination with the application of the conductive gel.

Results

In this study, eleven queens were brought to a private vet clinic to detect pregnancy. The pregnancy was diagnosed by abdominal palpation, Ultrasonography, and relaxin test. Table (1) shows the results of the pregnancy diagnosis by different methods.

Table 1: Results of pregnancy diagnosis by Abdominal palpation, Ultrasonography, and Relaxin test.

Case No.	Abdominal palpation	Ultrasonography	Relaxin test
1	Positive	Positive	Positive
2	Positive	Positive	Positive
3	Positive	Negative	Negative
4	Positive	Positive	Positive
5	Positive	Negative	Negative
6	Positive	Positive	Positive
7	Positive	Positive	Positive
8	Positive	Positive	Positive
9	Positive	Positive	Positive
10	Positive	Positive	Positive
11	Positive	Positive	Positive

The results illustrated that there were 11/11 cases were detected as pregnant by abdominal palpation, while 9/11 cases reported as pregnant when detected by Ultrasonography and Relaxin tests. The percentage of pregnancy rates in this study was 81 %, The results showed that the efficiency of the relaxin kit was 100 % in conformation with ultrasonography (figure 3 and figure 4) for positive results. Relaxin kit was crucial to differentiating pregnancy from pseudopregnancy in the cat.

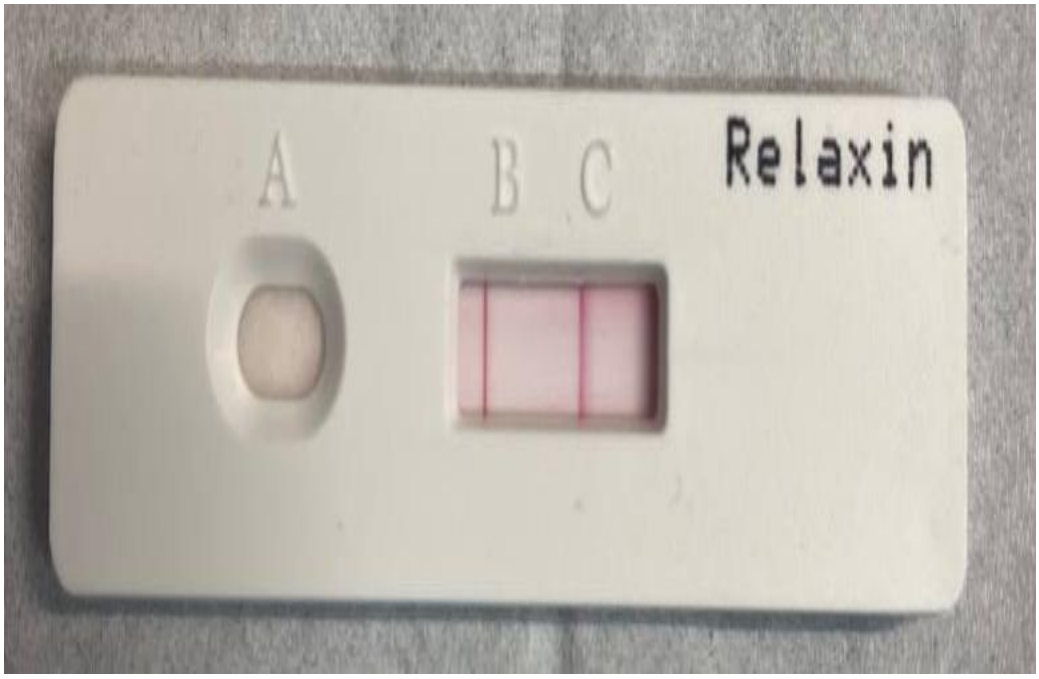

Figure 3: Positive results of Relaxin kit

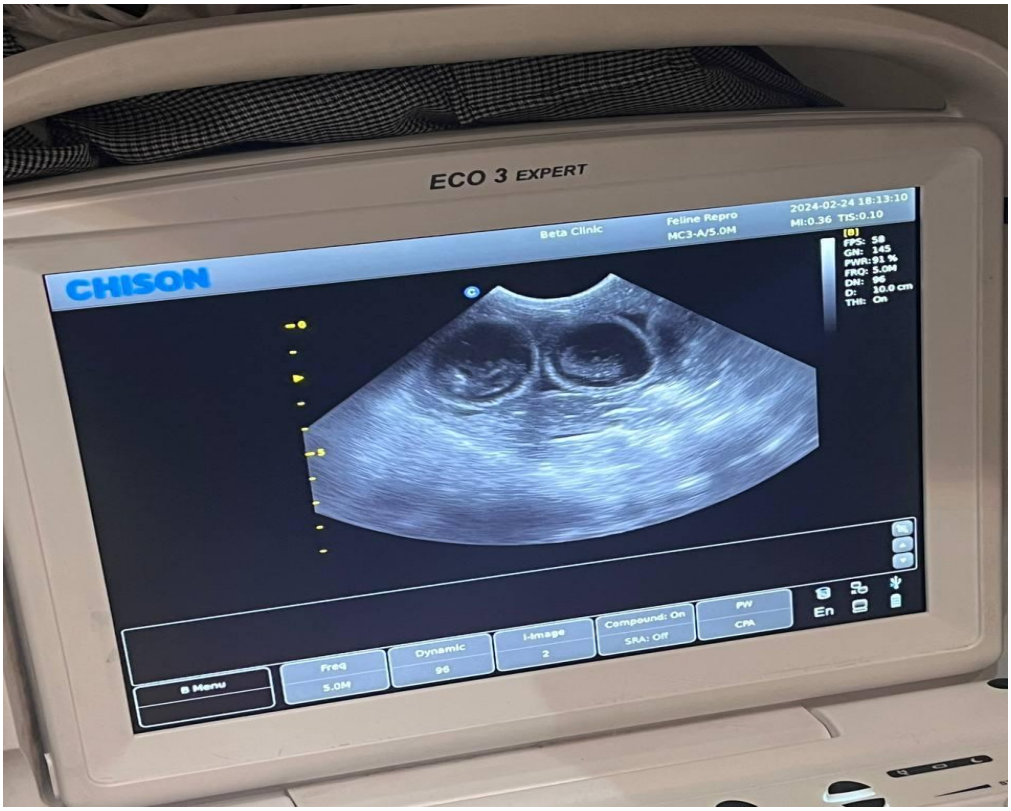

Figure 4: Ultrasonic image (5 MHz) for two feline gestational sacs.

Discussion

Relaxin is considered the only biological marker for pregnancy diagnosis in queen. The investigation of relaxin in this study was the first carried out as a hormonal method for pregnancy diagnosis in queens in Iraq and the specificity was 100 % as confirmed by ultrasonography. These results were in agreement with other studies (Schone *et al*., 2004, Bergfelt *et al*., 2014). Plasma concentration of relaxin can be noticed in cats from day 20 of pregnancy onward (DiGangi *et al*., 2010). Relaxin in urine can also be diagnosed from 21 to 28 days of pregnancy (Van Dorsser *et al*., 2007). The accuracy of abdominal palpation in this study was 81% and it coordinated with (Roos and Fontbonne, 2022), while (Johnston *et al*., 2001, Levy and

England, 2010) reported that it was difficult to palpate a fetus between 35 and 55 days after mating, conceptuses become elongated, enlarged and they lose their tenseness.

Davidson et al. (1986) was the first one who use ultrasonography for pregnancy diagnosis in cats. The use of ultrasonography to diagnose pregnancy in cats is the best method to offer an early diagnosis, a litter count (if done at early stages), and assess the vitality of fetuses. The results of our study conveyed that the using of ultrasonography for pregnancy diagnosis and monitoring of gestation in cats was effective and mostly distributed in private clinics in Hilla City during the last years, in which ultrasonography is used to detect pregnancy as early as 10 to 11 days after mating, as well a heartbeat can be indicated after 16 days of pregnancy (Zambelli *et al*., 2002 and Topie *et al*., 2015).

Conclusions

We conclude that using of Relaxin rapid test for pregnancy diagnosis in cats was accurate when made after 25 days of gestation with confirmation with ultrasonography.

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