

EARLY PREGNANCY DIAGNOSIS IN THE RABBIT BY REAL TIME ULTRASONOGRAPHY

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ABSTRACT : An ultrasonographic method for the diagnosis of early pregnancy in the rabbit is presented. New Zealand White does were artificially inseminated and their uteri were ultrasonographically examined on days 7, 8, 9 and 10 post insemination. On day 7, a low echogenicity area, located in the uterine lumen, could be regarded as an indication of

pregnancy. However, on day 8, embryonic vesicles with a diameter of about 8 mm were identifiable and countable. On day 9, embryonic vesicles developed up to a diameter of about 12 mm. Finally, on day 10, embryonic vesicles' diameter was around 17 mm and their embryonic pole was also identifiable.

RESUME : Diagnostic précoce de la gestation chez la lapine par échotomographie

Une méthode de diagnostic précoce par ultrason de la gestation chez la lapine est présentée. Des lapines Néo Zélandais Blanc ont été inséminées artificiellement et leurs utérus ont été examinés par ultrason 7, 8, 9 et 10 jours après l'insémination. Au jour 7, un faible écho dans la lumière utérine

peut être considéré comme une indication de gestation. Le 8^{ème} jour, des vésicules embryonnaires d'environ 8mm de diamètre peuvent être identifiés et comptés. Le 9^{ème} jour, les vésicules embryonnaires ont atteints 12mm. Enfin, au 10^{ème} jour les vésicules embryonnaires ont atteints un diamètre d'environ 17mm et leurs pôles sont identifiables.

INTRODUCTION

Ultrasonography has been widely applied for the diagnosis of early pregnancy in domestic animals [cow (WHITE *et al.*, 1985, SARATSI *et al.*, 1993), mare (McKINNON *et al.*, 1993), sheep (WHITE *et al.*, 1984), goat (HAIBEL, 1988), sow (INABA *et al.*, 1983), bitch and queen (BURKE and BADERTSCHER, 1986)], the last years. This useful technological innovation allows early, accurate and practical detection of pregnancy.

Rabbit pregnancy is traditionally diagnosed by palpation of the uterus through the abdominal wall on days 12-14 post coitum (p.c.) or days 10-12 p.c. by experienced practitioners (HAGEN, 1974). ELISA kits for bovine progesterone (Ovucheck ELISA kit / Cambridge Life Sciences) have also been used to measure rabbit plasma or serum progesterone concentration on day 17-18 p.c. in order to differentiate pregnant from pseudopregnant animals (MORRELL, 1990, 1993).

In this paper we present the technique together with the findings of the ultrasonographic examination of the uterus of pregnant rabbits on days 7, 8, 9 and 10 post insemination (p.i.) (day 0 : day of insemination).

MATERIALS AND METHODS

Sexually mature female New Zealand White rabbits were used. They were housed individually in wire cages, fed a balanced diet and subjected to a 16 hour photoperiod.

The does were artificially inseminated with fresh diluted semen to accurately estimate the stage of pregnancy during the study. At the time of insemination,

the does received an intramuscular injection of 20 µg of GnRH synthetic analogue (Gonadorelin, Ovarelin / Sanofi - CEVA).

The uteri of each animal were examined ultrasonographically on days 7, 8, 9 and 10 p.i. using a realtime ultrasound machine (Scanner 200 Vet / Pie Medical) equipped with a 5 MHz sector transducer. Pregnancy and non-pregnancy were confirmed by palpation of the uterus on day 12 p.i. and by checking kindling records.

Technique of the ultrasonographic examination of rabbit uterus

The ultrasonographic examination of the rabbit uterus is performed transcutaneously. The probe is positioned externally against the abdominal wall, while the doe is restrained at dorsal recumbency in a restraining device. It is imperative, for optimal image quality, that the space between the probe and the

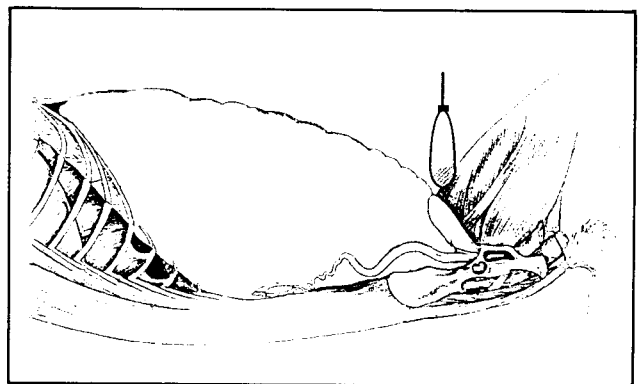


Figure 1 : Position of the probe at the beginning of the ultrasonographic examination of the rabbit genital tract.

RESULTS AND DISCUSSION

7th day of pregnancy

During the first sonographic examination, on day 7 p.i., no definite image of gravid uteri could clearly be seen. However, an area of low echogenicity, located in the uterine lumen, could possibly be regarded as an indication of pregnancy (Figure 3). Rabbit blastocysts enter the uterine lumen by 72-75 hours p.c. (LEWIS and GREGORY, 1929); however, on day 7 p.c., their diameter ranges from 4.5 to 5.9 mm (DANIEL, 1964) making their sonographic detection difficult.

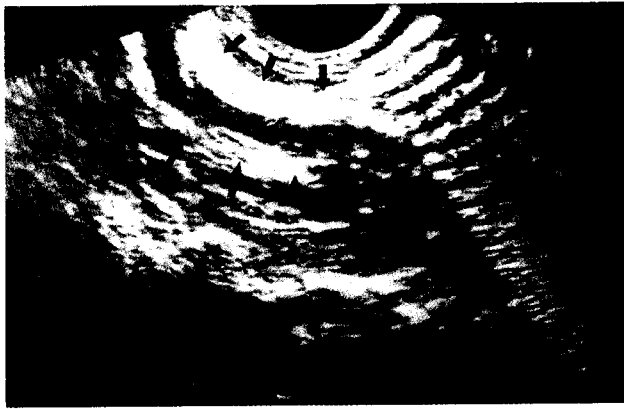


Figure 2 : Longitudinal section of rabbit vagina. The outline of the vagina is demarcated by the arrows. Ultrasonogram produced with sector transducer at 5 MHz.

animal's skin is free of air. For this reason, prior to scanning, the ventral abdomen of the doe is clipped and ultrasound scanning gel is applied at the examination site.

The sonographic examination proceeds from caudal to cranial. The probe is positioned directly in front of the pubic bone and held in a sagittal plane (Figure 1). At this position, the image of the long vagina can be seen, in a longitudinal section, as an echogenic linear tubular structure (Figure 2).

The probe is then moved cranially, following the course of the uterus, while its beam is directed either longitudinally or transversely to it. Due to its small diameter, it is almost impossible to sonographically distinguish a non gravid uterus, at a cross section, among the intestinal gyri.

The total time needed for the sonographic examination (restraint, clipping, examination) does not exceed 5 to 10 min. Clipping takes a few minutes longer when non-lactating does are to be examined, since more hair is present on their ventral abdomen.



Figure 4 : Transverse section of left and right rabbit uterus on the 8th day of pregnancy. The arrows point to embryonic vesicles. The left embryonic vesicle has a diameter of 8 mm, while the other one measures 10 x 6 mm (mean diameter : 8 mm). Ultrasonogram produced with sector transducer at 5 MHz.



Figure 3 : Transverse section of rabbit uterus on the 7th day of pregnancy. The outline of the uterine horn is demarcated by the arrows. A low echogenicity area is detected in the uterine lumen. Ultrasonogram produced with sector transducer at 5 MHz.



Figure 5 : Longitudinal section of rabbit uterus on the 9th day of pregnancy. The arrows point to three embryonic vesicles with diameters of 12 mm. Ultrasonogram produced with sector transducer at 5 MHz.

8th day of pregnancy

On day 8 p.i. the fluid filled embryonic vesicles were identifiable as dark anechoic round structures with a diameter of about 8 mm (Figure 4) which is within the limits (8-9.5 mm) reported by DANIEL (1964). The blastocysts were assumed to be perfect spheres, although sometimes they are slightly compressed along one axis. It was also possible, after a close examination, to count the number of conceptuses.

9th day of pregnancy

On day 9 p.i., embryonic vesicles reached a diameter of about 12 mm (Figure 5). Blastocyst development made ultrasonographic detection of pregnancy more accurate and easy. At this stage, diagnosis of pregnancy was beyond any doubt.

10th day of pregnancy

Finally, on day 10 p.i., embryonic vesicles expanded up to a diameter of about 17 mm. The echogenic thickened uterine wall surrounded the anechoic embryonic vesicles. Their embryonic pole could also be distinguished (Figure 6).

At this stage of pregnancy, ultrasonographic findings could be confirmed by palpation of the uterus or *vice versa*.

Differential diagnosis

It would be useful to differentiate the image of an embryonic vesicle from that of a ball of faeces, due to their similarity in shape and size, especially on days 9 and 10 p.i. or p.c.. Faeces usually have a diameter between 13 and 20 mm, but most importantly are echogenic due to their dense composition (Figure 7). The diameter of embryonic vesicles, on the other hand, ranges from 10.5 to 13 mm on day 9 and 10 to 20 mm on day 10 p.c. (DANIEL, 1964). They are structures containing transparent fluid, so they are anechoic and produce black images surrounded by the echogenic trophoblastic layer and the thickened uterine wall.

It is apparent that the rabbit uterus is ultrasonographically detectable only in pregnant animals. Embryonic vesicles are the structures that can be definitively detected. Although the first indication of pregnancy is visible by day 7 p.i., diagnosis can be made on day 8 p.i. In relevant studies the gravid rabbit uterus could clearly be demonstrated as early as day 9 of pregnancy (INABA *et al.*, 1986, TAINURIER *et al.*, 1986).

Apart from the clarity of the embryonic vesicles' image, the accuracy of diagnosis is another important aspect of the method. In a study involving a limited number (20) of rabbits, the percentage of correct diagnosis increased from 83% on day 8 to 100% on day 9 of pregnancy (INABA *et al.*, 1986). Nevertheless, we believe that an extensive evaluation of the accuracy of the



Figure 6 : Transverse section of rabbit uterus on the 10th day of pregnancy. The black arrow points an embryonic vesicle with a diameter of 17 mm, while the white arrow points its embryonic pole. Ultrasonogram produced with sector transducer at 5 MHz.

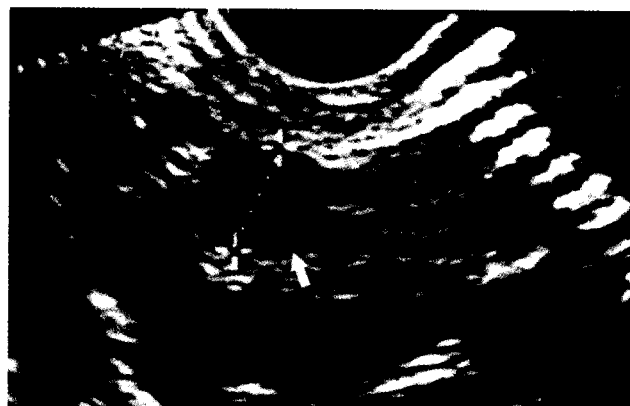


Figure 7 : Transverse section of rabbit large intestine. The arrow points a ball of feces with a diameter of 13 mm. Ultrasonogram produced with sector transducer at 5 MHz.

method should be made before recommending it in commercial rabbit farms.

The method could also be applied in rabbit embryo viability studies (teratology studies), although the accuracy of detection of the number, and probably the place, of implanted embryos would have to be further studied. Undoubtedly, in the biomedical research area, where expensive, time-consuming and complicated protocols are involved, early pregnancy diagnosis by ultrasonography in laboratory rabbits can be a valuable tool in the hands of the researcher.

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