



Sonographic Diagnosis of Pregnancy and Study of Gestational Changes in Rabbit-Does

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SUMMARY

This study was carried out to evaluate early pregnancy diagnosis using ultrasound and baseline information on the sonographic features of the reproductive cycle of rabbit-doe. Eight adult does, that had kittened at least once and an Ultrasound machine (Medison S600V[®]) with a 6.5 MHz transcutaneous curve-linear probe, were used for the study. Rabbit-does were mated naturally by the introduction of a doe to a buck. Abdominal regions were shaved liberally from the level of xyphoid cartilage to the pelvic area and aquasonic gel applied. The uterus was scanned on day 5 post-coitus and thereafter on days 7, 12, 15, 20, 25, 27 and 29, using the bladder as a land mark. Embryonic vesicles, visualized as small anechoic (darkened) structures were first seen on day 7 of gestation. Hypoechoic structures within vesicles corresponding to embryo and placenta were seen on day 12 with an increase in size at day 15 of gestation. Bony formation, bi-parietal diameter, vertebrae column, fetal heart and fetal heart rate were visible with progressive gestational age. This study demonstrated that ultrasound can be used effectively to diagnose pregnancy in rabbit-doe as early as day 7 of gestation. Also there is a correlation between the sonographic observable changes with gestational age.

Key words: Ultrasonography, Rabbit, Pregnancy Diagnosis, Gestation.

INTRODUCTION

Ultrasound (US) is an oscillating sound pressure wave with a frequency greater than the upper limit of the human hearing range (Novelline, 1997). Ultrasonic imaging (ultrasonography) is used in both veterinary and human

medicine (Novelline, 1997). US is one of the most widely used imaging technologies in medicine, it is portable, readily available, free of radiation risk, and relatively inexpensive when compared with other imaging devices (Edler and Lindstrom,

2004). Furthermore, US images are tomographic, i.e., offering a “cross-sectional” view of anatomical structures (Edler and Lindstrom, 2004). The frequencies used in medical imaging is between 2 and 10 megahertz (MHz), where 1 MHz is 1 million cycles per second, 50 times greater than the maximum frequency of audible sound by the human ear - hence the name *ultrasound* (Luc, 2010). US imaging is based on the 'pulse-echo' principle in which a short burst of ultrasound is emitted from a transducer and directed into tissue (Burns, 2011).

Reproductive ultrasonography has increased the knowledge of the changes during early pregnancy in different animals (Ginther and Pierson, 1984; Curran, 1986). With the use of a real-time, B-mode ultrasonography and 5 MHz transducer, pregnancy can be detected as early as 9 to 12 days post artificial insemination into gestation (Ginther and Pierson, 1984; Curran, 1986). Ultrasonography has been widely applied for diagnosis of early pregnancy in domestic animals such as cow (White *et al*, 1984, Seratsiset *al*, 1993), mare (McKinnon *et al*, 1993), sheep (White *et al*, 1984), goat (Haibel, 1988), sow (Inabaet *al*, 1983), bitch and queen (Burke and Bardertscher, 1986). The useful technological innovation allows early, accurate and practical detection of pregnancy (Ypsilantis and Saratsis., 1999). Rabbits do not have well defined oestrus cycle and they are often, erroneously, considered to be permanently in oestrus. Rather a period of receptivity occurs every 5–6 days (Capello, 2005). A doe is said to be in oestrus when she accepts service and in dioestrus when she refuses (Lebaset *al*, 2014). Like cats and ferrets, female rabbits are induced ovulators (Capello, 2005). Ovulation is normally stimulated by coitus and occurs 10 - 12 hours after mating (Lebas *et al*, 2014) and are usually fertilized about an hour and a half after their release (Quintela *et al*, 2004).

MATERIALS AND METHODS

Animals

Three (3) adult bucks and Eight (8) mature non-gravid Dutch breed of rabbit-does, which had kiddened at least once and at most thrice were used for the study. They were purchased from a reputable rabbit breeder in Samaru Zaria, Kaduna State, Nigeria. Their average age was 1½ years and average weight of 2 kg. They were housed in individual woodenmade clutches in the animal pen of the Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. They were fed with pelletized grower feed (Vital Feeds Nigeria Limited) containing 15% Crude Protein, 7% Fat, 10% Crude Fiber, 1% Calcium, 0.35% Phosphorus, 2,550kcal Metabolizing Energy and water provided ad libitum.

Equipment and consumables

Ultrasound machine (Medison S600V[®]) with a 6.5MHz transcutaneous curve-linear probe and a Sony videographic thermal printer with printing paper, aquasonic gel, tissue paper, cotton wool, antiseptic soap, packets of tiger razor blade, petrol and 1800 MW generator and extension cable.

Natural mating

Mating was achieved by the introduction of a doe into the clutch of a buck and allowed together for an average of 30 minutes. Afterwards the does were removed and returned to their respective clutches. Doe is diagnosed to be in oestrus when she accepts service and in diestrus when she refuses.

Ultrasound examination

Each doe was properly restrained physically and placed on dorsal recumbency. Fur from the level of the xyphoid cartilage, down to

the pelvic region was gently made wet, with a soaked cotton wool in water and antiseptic soap applied. The fur was then liberally shaved. The shaved region was cleaned thoroughly with a dry cotton wool and swabbed with wool soaked in antiseptic solution. Aquasonic gel was then applied on the skin. A portable ultrasound machine with a 6.5 MHz transcutaneous curve-linear probe was used to scan the abdomino-pelvic

region using the bladder as a landmark. The probe was placed gently on the skin, transversely and tilted longitudinally until a descriptive echographic image was achieved on the screen. This process was carried out on Day 5 post coitus and thereafter on days 7, 12, 15, 20, 25, 27 and 29. Upon getting a descriptive echographic image, the freeze button on the keyboard was used to freeze the echographic image, which was then



Plate I: Sonogram at day 7 of gestation showing embryonic vesicles (red arrows)



Plate II: Sonogram at day 12 of gestation showing conceptus (red arrow) and amniotic fluid (blue arrow)

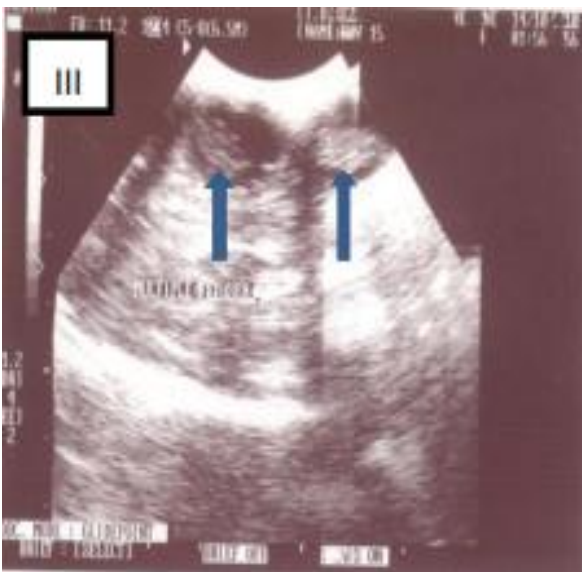


Plate III: Sonogram at day 15 of gestation showing multiple fetuses (blue arrow)



Plate IV: Sonogram day 25 of gestation showing bi-parietal diameter measuring 18mm (blue arrowed)

printed on thermal paper for documentation.

RESULTS

There was no suggestive sonographic sign of pregnancy at gestational day 5. However, embryonic vesicles (EV) were seen at gestation day 7 (Plate I). These structures appear as fluid filled round anechoic (darkened) structures measuring 6 - 9 mm (7.25 ± 1.035mm) in diameter with surrounding isogenic area. On day 12 of gestation, hypochoic structures were seen to occupy one-third of the embryonic vesicles, which corresponded to the placenta formation. The embryo and the remaining two-third EV, appeared anechoic which corresponded to the amniotic fluid nourishing the embryo (Plate II). Hypochoic structures and amniotic fluid within the vesicles, occupied about two-third and one-third of the vesicle respectively on day 15 of gestation (Plate III). Also, at this stage individual vesicles could be isolated and number of fetus (es) estimated. Hyperechoic structures within the uterus

depicting bone formation (BF) were seen on day 20. However independent bone structures could not be properly identified due to overlapping and size of fetus (es). Bi-parietal diameter (BPD) (Plate IV) and fetal heart (FH) were also visible at this stage as fetal heart rate could also be estimated (Plate V). BPD ranged between 18 and 29mm (21 ± 2.56mm) and the fetal heart beat (FHB) between 114 and 126 beats per minute (120.87 ± 3.97bpm) (Table 1). The total time needed for sonographic examination (restrain, clipping and examination) of a doe did not exceed 5 – 10 minutes. Although, clipping took few minutes longer when non-lactating does were to be examined.

DISCUSSION

The ultrasound result showed that the earliest time for detection of pregnancy in rabbit doe is on the 7th day of gestation evidenced by round dark areas that were anechoic, ranging from 6 – 9 mm in diameter surrounded by isogenic area denoting embryonic vesicles which were the

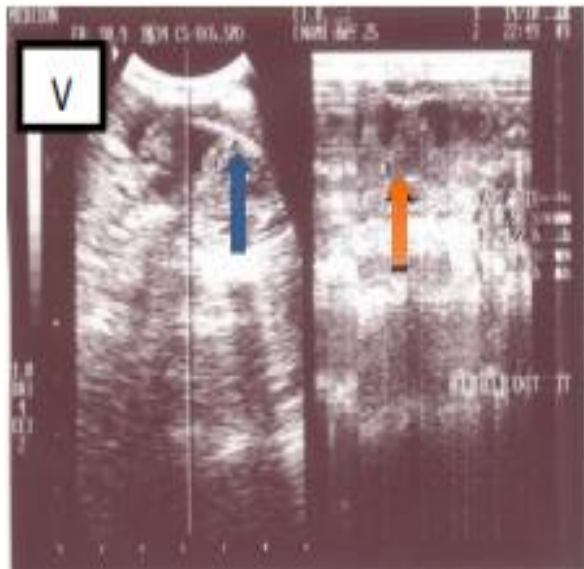


Plate V: Sonogram at day 25 of gestation showing vertebrae column (blue arrow and fetal heart rate reading 125beats/minute (red arrow)

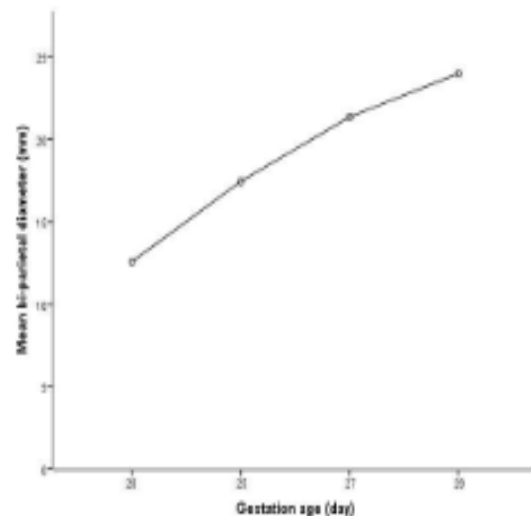


Figure 1: Variation of bi-parietal diameter with gestation age

Table 1: Sonographic parameters

Does	EVL	BPDL	FHB
1	7	19	120
2	6	18	118
3	9	23	126
4	8	20	120
5	7	18	125
6	8	22	124
7	6	29	120
8	7	24	114
Range	6 – 9	18 – 29	114 -126
Mean \pm SE	7.25 \pm 1.305	21 \pm 2.56	120.87 \pm 3.97

Key:

EVL: Embryonic Vesicle Length (mm)

BPDL: Bi-Parietal Diameter Length (mm)

FHB: Fetal Heart Beat (beats/minute)

first suggestive signs of pregnancy. This result is in agreement with the report of Ypsilantis and Saratsis (1999), Guteirrez and Zamora (2004) and Soroori *et al* (2008).

Embryonic vesicles and their progressive change in sizes (6 – 9 mm in diameter) characterized the first trimester in does. Second trimester however was characterized by placenta formation and development of the embryo within the amniotic fluid at the early stage seen in the study by Guteirrez and Zamora (2004). The beginning of bone formation which appears as hyperechoic structures in-utero marked the late stage of the second trimester. Most appreciable observable sonographic changes were seen during the third trimester because of the rapid growth and development at this stage. This is in agreement with the findings of Ajadi *et al* (2015). The fetal heartbeat detected as an anechoic pulsating oval shaped structure which is useful in the assessment of fetal viability also agrees with findings of Ajadi *et al* (2015). An increase in size of bi-parietal diameter, vertebrae column and appendages marked this stage of the cycle with an increase in gestational age. The increase in bi-parietal diameter with an increase fetal age agrees with the findings of Soroori *et al* (2008) and Nwaogu *et al* (2010) in rabbit and goats respectively where they both observed high significant correlation between bi-parietal diameter and fetal age.

It was difficult to associate structures to individual fetuses. This is due to an increase in fetal movement, thus overlapping of osseous structures such as vertebrae column and appendages as seen in a previous study by Guteirrez and Zamora (2004).

In conclusion, pregnancy in does can be confirmed as early as the 7th day post coitus and there is a good correlation between sonographic changes with gestational age.

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