



## Early Pregnancy Diagnosis using Trans-Abdominal Ultrasonography in West African Dwarf Goats

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### SUMMARY

The purpose of this study was to determine the earliest time pregnancy could be detected and the accuracy of pregnancy diagnosis in the West African Dwarf goat using trans-abdominal B-mode real-time ultrasonography. Seventeen does of varying parities (allotted to 2 groups; group 1 = 12 and group 2 = 5) and a buck of proven fertility were used for this study. The group 1 does were hand-mated following synchronized estrus while does in group 2 were left in the company of the intact fertile buck. Trans-abdominal scanning using an ultrasound machine equipped with a transducer of multiple frequency (5.0 to 8.0 MHz) was carried out every day in the group 1 does starting from Day 15 (Day of estrus/ breeding = day 0 of gestation) to Day 40 and, thereafter, every other day to Day 60 of gestation. Ultrasound scan of the group 2 does was undertaken randomly until confirmed pregnant. Acoustic coupling gel, Wavelength<sup>®</sup> was liberally applied on the animal skin area to be scanned. Sonograms were printed using UP 897MD thermal printer on Sony ultrasound paper; UPP110S. The earliest sonographic evidence of pregnancy was the imaging of circumscribed anechoic fluid in the uterus (EV) on Day  $18.8 \pm 0.29$  and the embryo on Day  $20.2 \pm 0.24$ . Heartbeat was detected in the embryos on Day  $23.8 \pm 0.91$ , embryo cephalization and development of limb buds on Day  $31.4 \pm 0.88$ , and the appearance of placentomes on Day  $34.4 \pm 0.42$  of gestation. Fifteen (ten from group 1 and all 5 from group 2) does (88.24%) were diagnosed pregnant by ultrasonography. All pregnant does subsequently kidded. The kids were born alive with no apparent morphological abnormalities. The computed average gestation length using the group 1 does was  $144 \pm 0.12$  days.

**Key words:** Pregnancy detection, accuracy, ultrasound, WAD goat.

### INTRODUCTION

Modern livestock farming increasingly uses reproductive biotechnology to improve animal production. This investment in technology in turn increases the need for early and accurate pregnancy diagnosis. Economic losses resulting from long inter-

kidding intervals, loss in kids and milk can be minimized when routine pregnancy diagnosis is made a part of herd reproductive management. This is because most reproductive failures are due to death during pregnancy or due to pregnancy

expectation in females with false pregnancy (Smith *et al.*, 1995). A practical method for early and precise detection of pregnancy has implication for profitable animal production (Santos, 2003; Dias *et al.*, 2008). Early pregnancy diagnosis is a management tool for the identification of non-pregnant animals that can be rebred with minimal delay (Yotov, 2005), treated or culled. In domestic ruminants, a number of methods for pregnancy diagnosis have been reported (Ishwar, 1995; Freitas and Simplicio, 2002; Santos, 2003). Some of these methods have limitations to their wide scale use in small ruminants (Wani, 1981; Goel and Agrawal, 1992; Gordon, 1999; Kharche and Kouamo, 2015). Some of these methods include rectal palpation which is limited by the small size of the pelvis of small ruminants (Wani, 1981) and the caudal artery monitoring, abdominal ballotment, non-return to estrus, and udder development which have had limited success (Wani and Sahni, 1980). The ultrasound machine is a modern and contemporary tool for routine pregnancy diagnosis that has become an integral part of reproductive technologies (Medan and Abd El-Aty, 2010; Karadaev *et al.*, 2015) used for the non-invasive visualization of the entire uterus (Streeter and Steep, 2007). B-mode real-time ultrasonography was introduced in the veterinary field and first used for pregnancy diagnosis in the mare (Palmer and Driancourt, 1980) and then received large acceptance for diagnosing pregnancy in all domestic animals (Kähn, 1992). Globally, sonographic diagnosis of different breeds of goats has been undertaken and shown to be accurate but there is paucity of information on the use of this technique in the West African Dwarf (WAD) goat. In Nigeria reports on the use of ultrasonography to determine the pregnancy status of goats are relatively novel and made within the last decade in Red Sokoto goats (Nwaogu and Anya, 2009; Nwaogu *et al.*, 2010; Omontose *et al.*, 2012). As veterinary ultrasonography becomes a tool for routine reproductive

management in Nigeria, this study was therefore conceived to evaluate its use for early pregnancy diagnosis in the West African Dwarf goat.

## MATERIALS AND METHODS

### Pre-experiment animal management

Seventeen female West African Dwarf goats of varying parities and a buck belonging to the experimental animal unit of the Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria, Nsukka were used for the study. The goats were routinely checked for both endo- and ecto-parasites and treated as appropriate. They were grazed on local pastures and forages during the day and given cassava and yam peels, bambara nut chaffs and kitchen green leafy vegetable wastes at night. Clean fresh water was provided *ad libitum*. The does were divided into 2 groups consisting of 12 for group 1 and 5 for group 2.

### Estrous synchronization and breeding

In the group 1 does, estrus was synchronized using cloproject<sup>®</sup> (0.025% cloprostenol; synthetic analogue of PGF<sub>2</sub> $\alpha$ ; KEPRO, BV, Holland). A double injection protocol 11 days apart of 250  $\mu$ g per doe of cloproject<sup>®</sup> was used. Following the second cloproject<sup>®</sup> injection (SCI), the does were housed in-doors, secluded from the buck and checked for estrous signs. Estrus detection was done using an intact virile buck four times a day at 06.00hr., 10.00hr., 14.00hr and 18.00hr, for 20 minutes each. Any doe that showed heat by standing to be mounted was allowed to be bred and the date of breeding recorded. One breeding only was allowed per doe and so date of breeding was considered Day 0 of gestation (Abdelghafar *et al.*, 2007). The group 2 does were run with the intact fertile buck and so date of fertile mating could not be recorded.

### Ultrasound scanning

Does for scanning were fasted overnight until scanning was over the next day. Each

doe was restrained in either lateral or dorsal recumbent position for scanning as described by Russel and Goddard (1995), in the relatively hairless region of the groin (Anwar *et al.*, 2008; Nwaogu and Anya, 2009; Nwaogu *et al.*, 2010) from one side to the other just in front of the udder. Sometimes during advanced gestation, it became necessary to clip some hair of the ventral abdomen. Copious amount of acoustic coupling gel, Wavelength<sup>®</sup> (National Therapy Products Inc., Canada), was applied on the probe head and skin of the doe in the area to be scanned. Trans-abdominal B-mode real-time ultrasound scanner, SA600V<sup>®</sup> (Medison Cooperation Limited, Seoul, Korea) equipped with a transducer of multiple frequencies (5.0 – 8.0 MHz) was used for the scanning. Sonograms were printed using a Sony printer, UP – 897MD on Sony thermal ultrasound paper, UPP 110S (Sony Corporation, Japan). Data generated included the earliest gestational age sonographic evidence of pregnancy was imaged; embryonic vesicle (EV), embryo proper, embryo with a beating heart, organization of the embryo into a head and limb buds and placentomes. Accuracy of pregnancy diagnosis was determined at kidding and expressed as a percentage.

## RESULTS

The result and accuracy of pregnancy diagnosis using ultrasound in the WAD goat is shown in table 1. In this study 17 does (12 does from group 1 that showed standing estrus and were bred and all 5 does in group 2) were subjected to ultrasound pregnancy diagnosis. Fifteen of the does (88.24%) were diagnosed pregnant while 2 does (11.76%) were diagnosed non pregnant. Using the group 1 does the earliest sonographic feature

of pregnancy was the imaging of circumscribed anechoic fluid-filled structure (EV) in the uterus on day  $18.8 \pm 0.29$  of gestation. The embryos proper were detected on day  $20.2 \pm 0.24$  as echogenic structures within the anechoic fluid in the uterus. All pregnant animals showed at least one embryo that typically appeared as an area of high echogenic density (Figure 2). Embryo heartbeat was detected on day  $23.8 \pm 0.91$  as hypoechoic area within the embryonic mass. Embryo cephalization and the appearance of limb buds were first detected on day  $31.4 \pm 0.88$  of gestation (Figure 3). Placentomes were first detected as small nodules on day  $34.4 \pm 0.42$  of gestation. As pregnancy progressed placentomes increased in size and appeared as C-shaped or O-shaped gray structures (Figure 4).

## DISCUSSION

In this study, the gold standard for pregnancy detection was that a doe diagnosed pregnant should kid after a normal gestation period. A doe was considered pregnant when a fluid-filled embryonic vesicle (EV), whole embryo/fetus, a fetal part, fetal membrane or placentome was recognized sonographically (White *et al.*, 1984; Buckrell *et al.*, 1986; Buckrell, 1988; Gunduz *et al.*, 2010). The overall accuracy of ultrasound in detecting pregnancy in the present study was 88.24% ( $15/17 \times 100$ ) and 100% for positive cases (Table 1). This percentage pregnancy was only computed following kidding. The percentage and accuracy of pregnancy diagnosis in the present study (88.24% and 100%, respectively) is similar to the 95.5% and 100%, respectively reported by Amer (2008) in Egyptian Baladi goats. Also, in

**Table 1:** The result and accuracy of pregnancy diagnosis using ultrasound in the WAD goat

	Number detected	Number correct	Accuracy (%)
Pregnant	15	15	100%
Non-pregnant	2	2	100%
Total	17	15	88.24



**Figure 1:** Early sonographic pregnancy diagnosis (circumscribed anechoic fluid (EV); day 18)

- EV- circumscribed anechoic fluid in the uterus

- UB- Urinary Bladder



**Figure 2:** Early sonographic pregnancy diagnosis (echoic embryo; Day 18)

E- Embryo

U- Uterine Lumen



**Figure 3:** Pregnancy Diagnosis Day 29 (Head and Limb Buds)

EF- Early Fetus

AM- Amniotic Membrane



**Figure 4:** Pregnancy Diagnosis; day 35

P = placentomes

this study the accuracy of detection of either pregnancy or non pregnancy by ultrasound was 100% (Table 1). This finding is in agreement with the reports of Medan *et al.* (2004), Amer (2008), Abdelghafar *et al.* (2007; 2009; 2010) and Gunduz *et al.* (2010) who found accuracies of 100% working with different breeds of goat. In the ewe, White *et al.* (1984) and Gearhart *et al.* (1988) found similar results. Ultrasonography is therefore,

an accurate and important tool for early pregnancy diagnosis in goats (Kahn *et al.*, 1990). In the present study the earliest evidence of conception was the imaging of circular or oval anechoic area within the uterine lumen that appeared black on the screen. These circumscribed anechoic fluid structures (EVs) were detected on day  $18.8 \pm 0.29$  after mating (Figure 1). The gestational age of detection of the EV

reported in this study is coming earlier than the reported age in some other studies (Padilla-Rivas *et al.* (2005), day 26.4; Amer (2008), day  $24.7 \pm 0.4$ ; Karen *et al.* (2009), day 27.87. The embryo proper appeared as defined echogenic structures within the anechoic fluid in the uterus (Figure 2). The presence of embryo was confirmed by day  $23.8 \pm 0.91$  with the imaging of a beating heart in a defined echogenic structure surrounded by a nonechogenic area of variable size (fluid) (Figure 2). The gestational age of detection of an embryo with a beating heart (day  $23.8 \pm 0.91$ ) in this study is coming earlier than the reported age in the works of some other researchers in the goat (Padilla-Rivas *et al.* (2005), day 33.1; Amer (2008), day  $27.0 \pm 0.6$ ; Karen *et al.* (2009), day 30.36. The reasons for the earlier gestational age of detection of evidence of pregnancy in this study when compared to the other studies were because of the earlier commencement of ultrasound scanning (day 15 in the present study versus day 25 in the other studies), the interval of ultrasound scanning (daily in the present study versus 3-5 day intervals in the other studies), transducer frequency used (Martinez *et al.*, 1998) (5.0 – 8.0 MHz in the present study versus 3.5 - 5.0 MHz in the other studies) and possibly breed differences (Karen *et al.*, 2009). It is reported that the higher the transducer head frequency (as was the case in the present study), the better the resolution or image quality (Buckrell, 1988). The penetration depth of ultrasound is however, reduced with increasing transducer head frequency (Herring and Bjornton, 1985), but the WAD goat is a smaller breed when compared to the breeds in the other studies and the distance the ultrasound waves have to travel to get to the gravid uterus is shorter than for the breeds in the other studies. The earlier age of detection of evidence of conception in this study is however, corroborated by the reported visualization of the conceptus (concept that includes the embryo or the fetus as well as the extra embryonic

membranes) from day 16 in goats and from day 19 in sheep (Santiago-Moreno *et al.*, 1995a, b; Gonzalez-Bulnes *et al.*, 1998) and in cattle as early as 9 (Boyd *et al.*, 1988), 10 (Curran *et al.*, 1986) or 12 days (Pierson and Ginther, 1984) of gestation. Also in cattle, Kastelic *et al.* (1989) monitoring pregnancy in heifers on day 18, 20 and 22 of gestation reported that the accuracy of pregnancy diagnosis improved to 85%, 100% and 100%, respectively. The earlier gestational age of confirmation of conception by visualization of embryo heart beat in the present study is also in agreement with studies in the ewe in which embryo heart beat was reported from day 18 (Schrick and Inskoop, 1993) to 30 (Buckrell *et al.*, 1986) and in goats on day  $20.7 \pm 0.5$  with a range of 19 to 23 (Martinez *et al.*, 1998) of gestation. In those studies just like in the present one the exact date of artificial insemination or natural mating was known and ultrasonography was simply used as a confirmation of pregnancy or to validate that detection of pregnancy was possible within the first three weeks of pregnancy. The idea in the present study was to detect pregnancy as early as possible and hence the early commencement of ultrasound monitoring. Because of the motivation to detect pregnancy as early as possible coupled with fasting of the does for 12 - 14 hours prior to scanning and shaving of the ventral abdomen it was possible to detect evidence of pregnancy (EV) as early as day  $18.8 \pm 0.29$  as reported in this study. This is in contrast to the usual field practice of trying to diagnose pregnancy around Day 40 of gestation. All does with detected EVs were subsequently confirmed pregnant by detection of embryo heart beat and by kidding after a normal gestation length. Fortunately no embryonic losses or abortions were recorded in this study thus, making the accuracy of detection of pregnancy as well as non-pregnancy 100%. In conclusion, transabdominal B-mode real-time ultrasonography has been shown to be

an accurate method for early pregnancy detection in West African Dwarf goats.

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