

THE IMAGE OF EMBRYO AND FETAL OF ETAWA CROSSBRED GOATS USING ULTRASONOGRAPHY AT DIFFERENT GESTATIONAL AGES

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ABSTRACT

This study aimed to estimate the gestational age of Etawa crossbred goats based on ultrasound images of embryo and fetal using transcutaneous ultrasonography. Etawa crossbred does ($n=5$) were synchronized using double PGF2 α injection with 10 days interval and mated naturally. Early pregnancy was diagnosed on 20th day after mating base on isoechogenic image of the embryo (2.65 ± 0.75 mm) and hypoechogenic appearance of gestational sac. On 30th day of the first trimester, the embryo size was 8.31 ± 0.42 mm. The embryo reached 10.44 ± 0.51 mm on 35th day. On 60th day, the existence of the fetal were clearly appeared along with isoechogenic of umbilical cord, hyperechogenic *os cranium* (25.5 ± 0.1 mm), and body length (51.83 ± 1.59 mm). On 75th day, the diameter of the *os cranium* was 33.03 ± 0.20 mm, body length of 110 ± 1.73 mm, and bones structures appeared hyperechogenic for *os thoracalis* and *os vertebrae*. The placenta was isoechogenic with diameter of 23.3 ± 0.2 mm. On 90th day, the placenta dominated the uterus (22.67 ± 1.70 mm) and reached its maximum diameter on 145th day (33.73 ± 2.91 mm). In conclusion, early pregnancy detection of Etawa crossbred goats using transcutaneous ultrasonography could be performed on 20th day after mating.

Key words: embryo, Etawa crossbred goats, fetus, transcutaneous ultrasonography

ABSTRAK

Penelitian ini bertujuan mengestimasi usia kebuntingan kambing Peranakan Etawa (PE) berdasarkan gambaran embrio dan fetus menggunakan ultrasonografi transkutan. Kambing PE betina induk ($n=5$) disinkronisasi dengan injeksi ganda PGF2 α dalam interval 10 hari dan dikawinkan secara alami. Kebuntingan dini dapat didiagnosis pada hari ke-20 pasca perkawinan dengan tampilan isoechogenic dari embrio ($2,65\pm 0,75$ mm) dan tampilan hypoechogenic dari gestational saccus. Trimester pertama hari ke-30 kebuntingan, embrio berukuran $8,31\pm 0,42$ mm. Ukuran embrio mencapai $10,44\pm 0,51$ mm pada hari ke-35. Pada hari ke-60 keberadaan fetus terlihat jelas, tampilan isoechogenic umbilical cord dan hyperechogenic *os cranium* ($25,5\pm 0,1$ mm) dan panjang tubuh ($51,83\pm 1,59$ mm). Hari ke-75 *os cranium* berdiameter $33,03\pm 0,20$ mm, panjang tubuh $110\pm 1,73$ mm dan struktur tulang dengan tampilan hyperechogenic dari *os thoracalis* dan *os vertebrae*. Hari ke-75 plasentom dengan tampilan isoechogenic berdiameter $23,3\pm 0,2$ mm. Hari ke-90 plasentom semakin mendominasi uterus ($22,67\pm 1,70$ mm) dan hari ke-145 diameter plasentom mencapai maksimum ($33,73\pm 2,91$ mm). Kesimpulannya adalah deteksi kebuntingan dini pada kambing PE menggunakan ultrasonografi transkutan dapat dilakukan pada hari ke-20 pascaperkawinan.

Kata kunci: embrio, kambing peranakan etawa, fetus, ultrasonografi transkutan

INTRODUCTION

Etawa crossbred goat is one of goat types that produce meat and milk developed in Indonesia which is considered having high economic value. From the price view, the milk of Etawa crossbred goat has higher price than cow milk (Sodiq and Abidin, 2008). The milk production of Etawa crossbred goat is 0.45-2.2/liter/goat/day. The lactation period length varies greatly (92-256 days) with average of 156 days. With good management, Etawa crossbred goat doe can produce milk over 200 days in a year. The average production of goat milk in Indonesia is lower (2-3 liter/goat/day) than other subtropical regions which can reach 5-6 liter/goat/day on highly productive Etawa crossbred goat (Sodiq and Abidin, 2008).

One of the purposes of the animal husbandry development is to increase the production of livestock to fulfil the domestic consumption need of meat and milk along with affordable price for society (Marzuki and Dwijatmiko, 2004). To meet the domestic consumption need, at least 5.6 billion goats/year is

needed, with the addition from foreign demands. According to Marzuki and Dwijatmiko (2004), in order to reach the animal husbandry development, there are three approach strategies in the form of technical approach, integrated approach, and agribusiness approach. Technical approach is interpreted as an effort to increase population number of livestock through number of births. Increasing production and population of livestock could be performed using quality improvement of reproduction either for male and female goats; therefore well operated management of livestock reproduction is essentially needed.

Early detection of pregnancy is one of important aspects in reproductive management and should be performed after the animal mate. Early detection of pregnancy can offer rapid information of mating success so that production efficiency due to infertility could be achieved and in turn would reduce the expense of breeding program and aid livestock management economically. Furthermore, faster evaluation and differentiation between pregnant and non-pregnant livestock could increase the efficiency of

reproduction and reduce the production cost (Gonzalez *et al.*, 2004, Karen *et al.*, 2004).

In foreign countries, transabdominal ultrasonography technique has been utilized along with great accuracy as diagnosis tool of pregnancy and estimation of the number of embryo in lamb (Buckrell, 1988; Garcia *et al.*, 1993; Anwar *et al.*, 2008), goat (Martinez *et al.*, 1998; Gonzalez *et al.*, 2004; El-Zarkouny *et al.*, 2008), and deer (Revol and Wilson, 1991). Amrozi and Setiawan (2011) reported that the use of ultrasonography could also be performed to observe the embryo and fetal development in order to estimate the gestational age of Garut lamb. Ultrasound image of certain organ from fetus could be utilized to estimate gestational age. The measurement of fetal body and head diameter obtain high accuracy in estimating gestational age and determining birth (Goddard, 1995).

In Indonesia, study of goat reproduction is very limited, particularly about the diagnosis of early detection and examination of gestational age using ultrasonography even though early information of pregnancy status may give plenty of benefit to the goat management and reproduction business. According to Heibel and Perkins (1989), the estimation of pregnancy diagnosis and gestational age is important to achieve maximum reproductive efficiency.

According to the previous descriptions, it is necessary to perform the study of ultrasonography usage toward the observation of embryo and fetal development in order to estimate the gestational age of Etawa crossbred goats.

MATERIALS AND METHODS

The sample used in this study was five female Etawa crossbred goats with productive age (2-4 years) with normal estrus cycle and one male Etawa crossbred goat as teaser. The estrus cycle of Etawa crossbred goats had been synchronized using double intramuscular injection of PGF2 α (1 mL/goat) with interval of 10 days. All Etawa crossbred goats were mated naturally when the estrus signs appeared. The diagnosis of early pregnancy and embryo fetal growth observation were performed using transcutaneous ultrasonography.

The Procedure of Transcutaneous Ultrasonography Usage

Ultrasonography tools (MINDRAY DP3300 VET, Shenzhen Mindray Bio-Medical Electronic Co. Ltd, China) was placed at the left side of operator which was placed at the left position of the goat. Scanning was performed using abdominal probe 3.5 Mhz (35C50EB, Shenzhen Mindray Bio-Medical Electronic Co. Ltd, China) when the doe goat was on laying position. Some part of the hairs were shaved before scanning to visualize best images, KY jelly was polished around abdomen in front of the mammae, probe was directed towards cranial of mammae until sebum which filled the inguinal gland, and then directed to dorsal and a little caudomedial. Probe was

slightly pressed on the abdomen towards urinary bladder. The interpretation of form, size changes, and detected organ were performed real time. Maximal picture of head diameter and fetal body length were searched and the measurement number appeared in the ultrasonography monitor. Sonograms were kept in digital picture form. On the ultrasonography monitor, the image of embryo, fetal, uterus, and bones showed white picture (hyperechogenic/hyperechoic), placental gave grey color (isoechogenic/hypoechoic), whereas amnion and uterus lumen showed black color (hypoechoic/anechoic). Fetal length was measured from forehead to tail base (Ali and Hayder, 2007) or peak of the head to heart then heart to the base of tail (Abdelghafar *et al.*, 2011). The observation of embryo and fetal growth using ultrasonography was performed in each trimester, which were in first trimester were performed in 14th to 20th day, subsequently in 30th day, and 35th day. Then in second trimester the measurements were performed in 60th, 75th, 90th day, and on third trimester were performed in day 120th and 145th of pregnancy.

Data Analysis

The observation data of ultrasound pictures of embryo and fetal growth of Etawa crossbred goats were analyzed descriptively.

RESULTS AND DISCUSSION

The results of five Etawa crossbred does which had been synchronized their estrus cycle using prostaglandin and mated naturally showed 100% positive pregnancy. Post mating observation using ultrasonography showed that early detection can be firstly detected in day 20th after mating, as reported in Figure 1. On the 14th to the 19th day the embryonic vesicle continued to develop, indicated by the appearance of hypoechogenic of embryonic vesicle fluid. According to Amrozi and Setiawan (2011), early indication of pregnancy is embryonic fluids inside the uterus. Study by Sayuti *et al.* (2016) showed that early pregnancy diagnosis in kacang goat could be firstly detected using transcutaneous ultrasonography on the 14th day after mating marked by hypoechogenic fluid from embryonic vesicle. Buckrell (1988) reported that hypoechogenic visualization starts to appear in embryonic vesicle on the 14th-19th days of pregnancy. Pregnancy could be marked by the development of embryo vesicle and the existence of fetal in uterus (Amrozi and Tumbelaka, 2014).

An animal is expected to be pregnant when it contains gestational sac, conceptus, cotyledon, and other parts of the fetal inside the uterus (Abdelghafar *et al.*, 2007; Amer, 2008; Anwar *et al.*, 2008). The image was obviously different between the uterus of pregnant Etawa crossbred goat and non-pregnant Etawa crossbred goat after mating (Figure 2). The uterus of non-pregnant Etawa crossbred goat showed isoechogenic uterus lumen, no hypoechogenic appearance from embryonic vesicle and no image of

isoechogenic embryo as early indication of diagnosing pregnancy.

Kahn (2004) reported that the early indication of pregnancy in goat could be observed in 12th day with the appearance of anechoic (black) embryonic fluid from embryonic vesicle and 24th day when the embryo can be firstly detected using transrectal ultrasonography method. This study showed that the embryo of Etawa crossbred goat could be detected using transcutaneous ultrasonography at 20th day. The embryo size was 2.65 ± 0.75 mm with isoechogenic appearance and gestational sac with hypoechogenic appearance. This result was obtained more quickly than when performed

on Kacang goats using similar ultrasonography technique. The isoechogenic embryo images in Kacang goat could be detected on the 24th day during pregnancy with embryo size of 6.77 mm (Sayuti *et al.*, 2016), and 26 days after mating on Boer goat (Rivas *et al.*, 2005). Martinez *et al.* (1998) reported that goat embryo could be detected on day 19th after mating using 5 MHz transducer. At 20th day after mating, early detection of pregnancy in lamb could be detected by the appearance of the embryo; moreover it will be more accurate when being detected at 25th day (Schrack and Inskeep, 1993), or 26th day (Buckrell *et al.*, 1986). Ishwar (1995) reported that early detection on lamb and

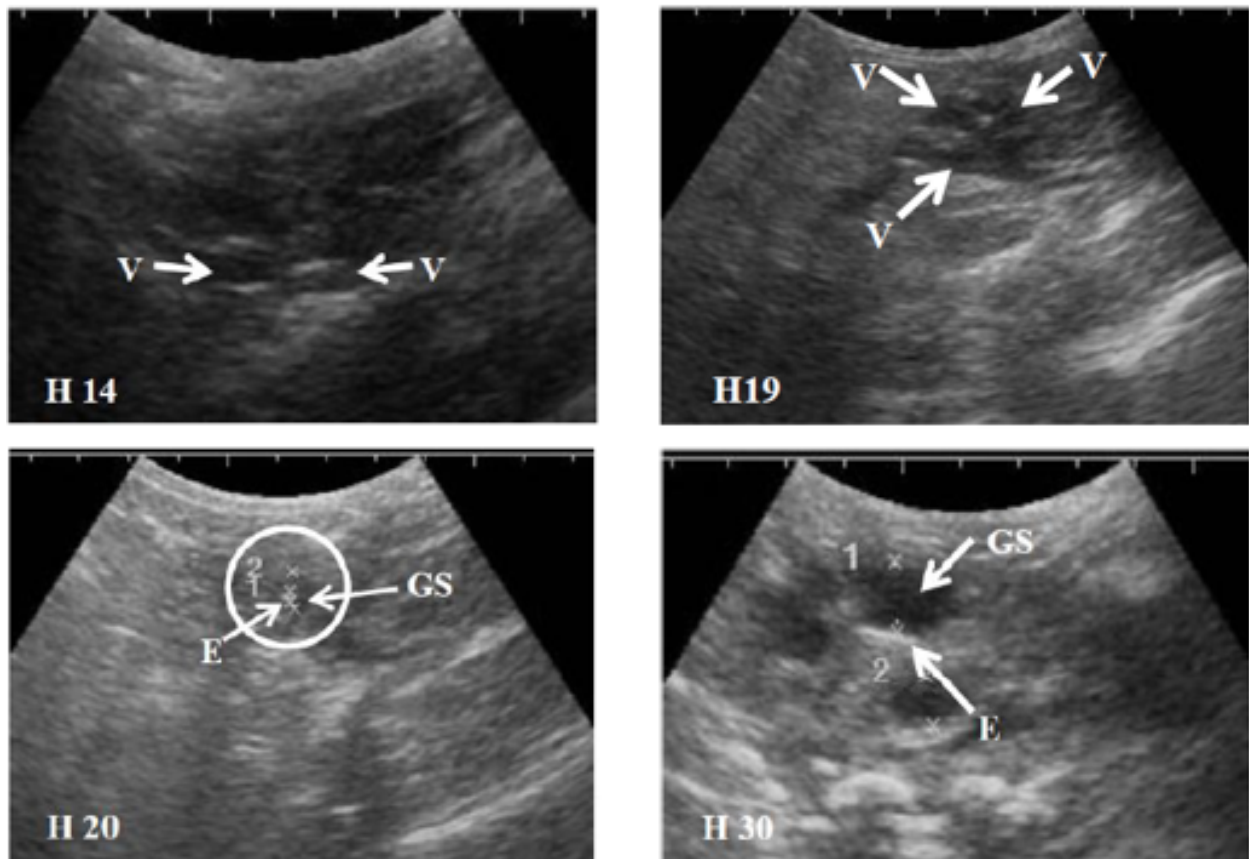


Figure 1. The transcutaneous ultrasonography image of the Etawa crossbred goat uterus after mating at the first trimester (H= Day after mating, V= Embryonic vesicle, E= Embryo, GS= Gestational sac)

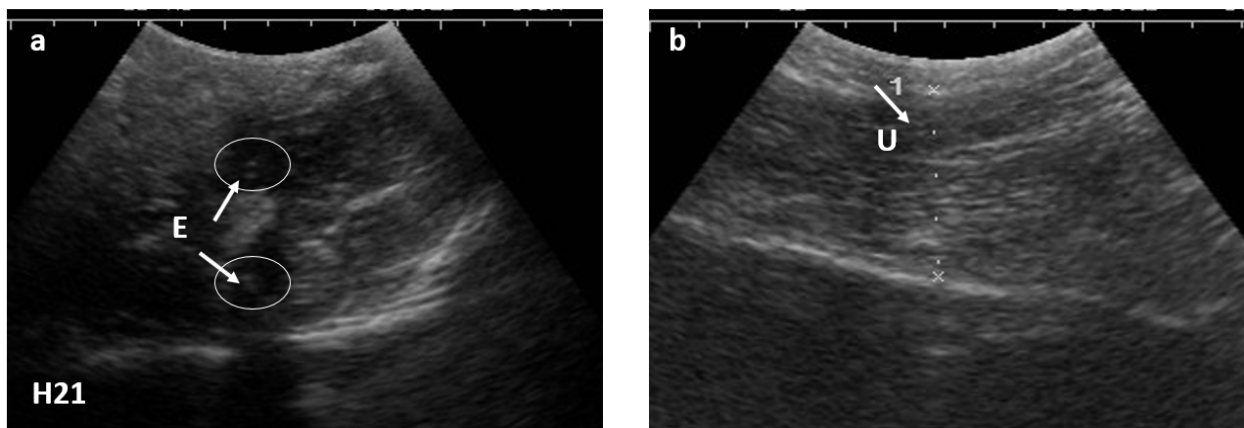


Figure 2. The image of transcutaneous ultrasonography of Etawa crossbred goat after mating. a= Pregnant (H= Day after mating, E= Embryo, GS= Gestational sac), b= Non pregnant

goat could be detected at the 25th day of pregnancy. According to Ali and Hayder (2007), length measurement of embryo and amnion sac on Ossimi lamb could only be detected at first trimester of pregnancy, in which embryo could be firstly detected between 24th-27th days of pregnancy.

Embryo continued to grow and started to increase in size. On 30th day of Etawa crossbred goat pregnancy, embryo with isoechogenic appearance had 8.31 ± 0.42 mm (Figure 1). The embryo size reached 10.44 ± 0.51 mm on 35th day of pregnancy (Figure 3). In the first trimester, embryo underwent development. According to Christensen (2015) goat embryo period starts in approximately 12th day when blastocyst attaches to the uterus layer and approximately 42th day when the embryo grows very fast so that it marks the end of the

embryonic period. Then, the embryonic period is replaced by the fetal period.

In the second trimester of pregnancy, on 60th day after mating, the existence of fetus was obviously appeared from the isoechogenic umbilical cord and hyperechogenic *os cranium* with diameter of 25.5 ± 0.1 mm and body length on its longest axis of 51.83 ± 1.59 mm which was surrounded by hypoechogenic appearance from the pregnancy fluid. On 75th days, the fetal growth was more obvious with the marked growth of *s cranium* which was 33.03 ± 0.20 mm and body length reached 110 ± 1.73 mm (Figure 4).

Furthermore, the bone structure was observed by the hyperechogenic appearance from *os thoracalis* and *os vertebrae*. Placentom with isoechogenic appearance had diameter of 23.3 ± 0.2 mm and was obviously

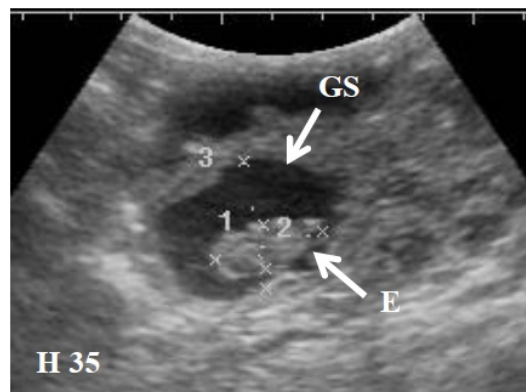


Figure 3. The transcutaneous ultrasonography image of Etawa crossbred goat pregnancy at 35th day (H= Days of pregnancy, E= Embryo, GS= Gestational sac)

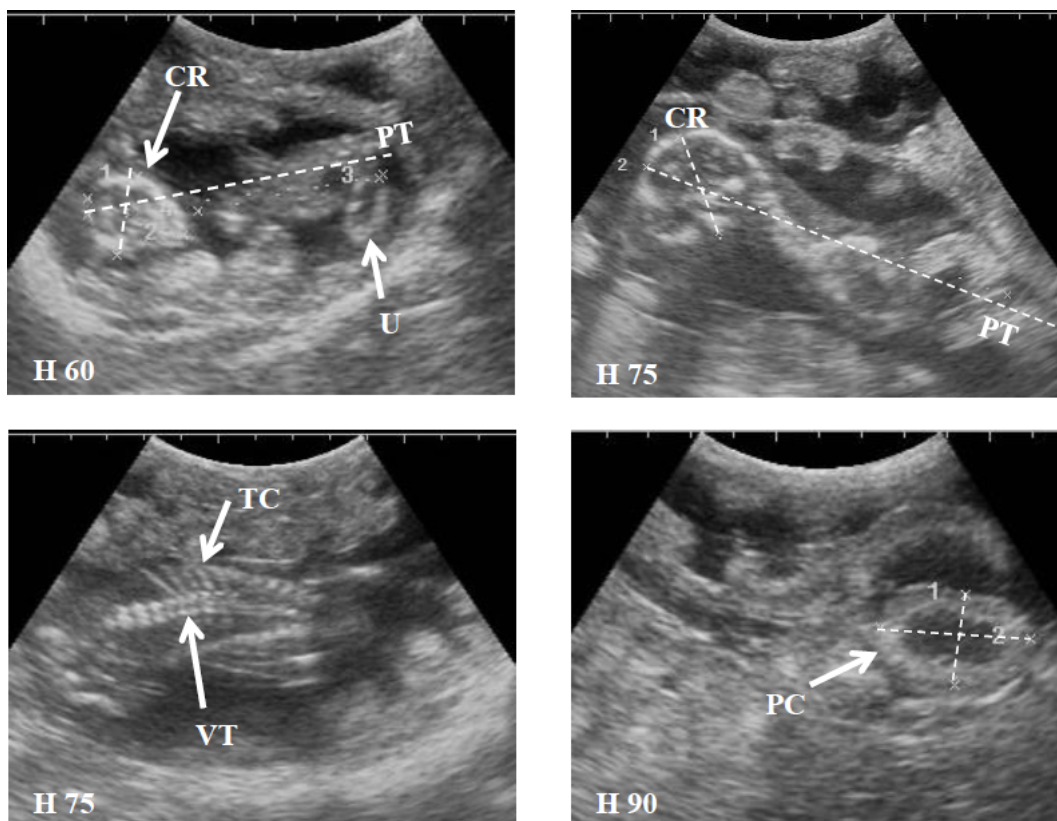


Figure 4. The transcutaneous ultrasonography images of Etawa crossbred goats pregnancy at second trimester (H= Days of pregnancy, CR= *Os cranium*, PT = Body length, U= *Umbilical cord*, TC= *Os thoracalis*, VT= *Os vetebrae*, CV= *Os cervicalis*, PC= Placentom)

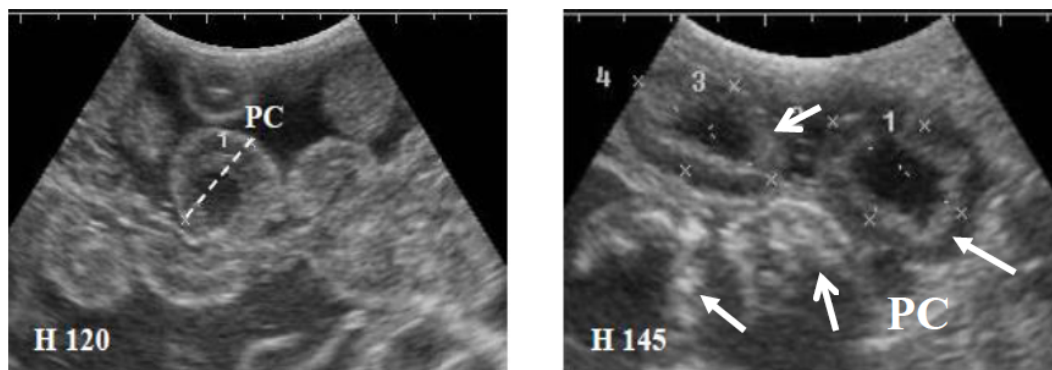


Figure 5. The hyperechogenic appearance of placentom dominating lumen uterus at the last trimester of pregnancy (H= Days of pregnancy, PC= Placentom)

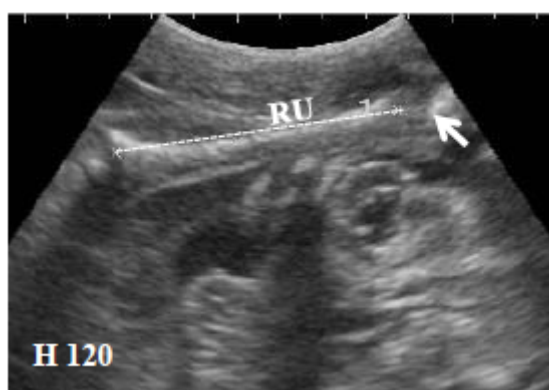


Figure 6. Hyperechogenic *os radius ulna* (RU) appearances of the fetus before born

observed on the 75th days of pregnancy, and got more dominating on the 90th days of pregnancy with size of 22.67 ± 1.70 mm. On the 145th days of pregnancy the placentom diameter reached its maximal size which was 33.73 ± 2.91 mm, as seen on Figure 5.

According to the observation of Abdelghafar *et al.* (2011), fetus length size of Saanen goat which was performed on 57th days after mating reached 8.73 cm. On the 42th-48th days of pregnancy, the head bone image had been observed, while on the 46th-55th days of pregnancy the vertebrae and costa bones were obviously appeared in Ossimi lamb (Ali and Hayder, 2007). The ossification and changing process of echoic images from turbid appearance of fetal fluid could be utilized to determine the age of pregnancy (Hafez, 2000).

Anwar *et al.* (2008), stated that placentom and leg bud were observed 100% between 45th and 50th days of pregnancy. Amrozi and Setiawan (2011) reported that the placentom of Garut lamb appeared on 34th days. Placentom showed development pattern which continuously increased in significant pattern until eventually the placentom growth became insignificant (relatively constant) after the 56th days. According to Buckrell *et al.* (1986) placentom could be detected using transrectal ultrasonography (5 MHz) on 30th and 32th days of pregnancy (Doize *et al.*, 1997). On the 40th days, the placentom could be identified on lamb marked by echoic circular structure (Russel, 1985). Doize *et al.* (1997) reported that lamb placentom reached its maximal size at 74th days and described as resembling a cup.

Ultrasonography observation at third trimester of pregnancy were performed at 120th and 145th days when at those days the fetal images were unclear due to increased size of the fetus and the placentom was dominating the lumen of the uterus (Figure 5). Beside to estimate the gestational age, ultrasonography could also be used to observe the fetal position. In this study, the normal/abnormal position of the goat could be identified at 120th days just before the birth through hyperechogenic *os radius ulna* appearances which getting more pointed to the birth canal (Figure 6).

CONCLUSION

Early detection of pregnancy in Etawa crossbred goat using transcutaneous ultrasonography can be performed at 20th days after mating. The gestational age estimation of the Etawa crossbred goat using transcutaneous ultrasonography can be determined by measuring the embryo length, body length of the fetus, diameter of the *os cranium*, and placentom.

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