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Research Article

Diagnosis and monitoring of pregnancy in banteng (*Bos javanicus*) by ultrasound

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ABSTRACT

Pregnancy can be detected by several methods, including rectal palpation, measurement of progesterone hormone concentration, and ultrasound (USG). The aim of this study is to diagnose early pregnancy and monitor pregnancy development using ultrasound in Javanese Banteng (*Bos javanicus*). Three female Banteng from Taman Safari Indonesia, Cisarua, Bogor, Indonesia, were used for this study. Pregnancy diagnosis was performed 11, 18 and 20 days after insemination. Diagnosis was based on the presence of the amniotic sac, heart rate, and embryonic and fetal development. Embryonic and fetal development were monitored until day 60 of gestation, and placental development was monitored from day 60 to day 270. The results showed that on day 20 after insemination, a 0.33-cm chorioallantois membrane with a white spot inside was detected. On day 27, a 0.55 cm embryo and a 1.16 cm amniotic sac were detected. The size of the placenta of the three bantengs was 1.21 ± 0.3 cm on day 60 and continued to increase until it reached 5.57 ± 0.26 cm on day 270. This study concludes that it is possible to diagnose early pregnancy at day 20 after fertilization and placental development in Banteng.

Key words: Banteng, Pregnancy diagnosis, Placentome, Ultrasound

INTRODUCTION

The importance of wildlife conservation is because they are an integral part of the ecosystem, also helps maintain overall environmental balance. Disturbances to wildlife populations can have negative impacts on ecosystems and human health. Banteng (*Bos javanicus* d'Alton 1823) is one of Indonesia's native germplasm that needs to be developed and conserved, it is an endangered species based on the IUCN red data list (Gardner *et al*., 2016). In the veterinary field, ultrasound is used to confirm the diagnosis of a disease (Samir *et al*., 2022). In the reproductive field, ultrasound is used to detect disease and reproductive disorders (Fontes and Oosthuizen, 2022). The use of ultrasound is widespread for screening in early pregnancy and monitoring fetal growth and development during pregnancy (Perry and Cushman 2016). Ultrasound also provides other advantages like determining the number of fetuses, fetal age and sex, fetal deaths, and monitoring fetal development (Gürler and Kaymaz, 2011). Souza *et al*. (2011) added that ultrasound can be used for rapid and noninvasive assessment of the reproductive tract and diagnosis of uterine status (Balhara *et al*., 2013).

In Bovines, early determination of pregnancy status is probably the most important factor in establishing reproductive performance. Early detection of open (non-pregnant) cows females enables better evaluation of their conception rate (which is often related to male or semen fertility), the effectiveness of an artificial insemination program, and possible underlying infectious/ non-infectious diseases (Burns *et al*.,2010). In addition, early detection of non-pregnant animals after mating is essential for optimal management of the calving season (Abdullah *et al*., 2014). Timely detection of pregnancy improves optimal reproductive management for each animal and maximizes profitability of dairy and beef farms (Gargiulo *et al*., 2012).

Ultrasonography can be used transrectally or transabdominally with high sensitivity (Aziz and Al-Watar, 2022). Ultrasound is defined as sound frequencies above the normal hearing range of the human ear, which is above 20,000 Hz. Rantanen *et al* (1981) reviewed, in detail, the manner in which ultrasound functions and offers a visually interpreted image. Ultrasound for pregnancy diagnosis is already widely used in livestock (Medan and Abd El-Aty (2010), pet animals such as dogs and cats (Davidson, 2022) as well as in wild animals (Hildebrandt and Saragusty, 2022). Semenov *et al*. (2014) reported pregnancy diagnosis using ultrasound in female Black Sea Bottlenose Dolphin (*Tursiops truncatus ponticus*), while Mc. Nay *et al*. (2006) reported pregnancy diagnosis, in utero litter size, and fetal growth in free-ranging wolves. No information is available on the detection and development of pregnancy in Javanese Banteng (*Bos javanicus* d'Alton 1823) as one of the wild animals. Therefore, the aim of this study is use ultrasound to diagnose early pregnancy and monitor thaty pregnancy in banteng until shortly before birth.

MATERIALS AND METHODS

Place and time of the study

This study was conducted at Taman Safari Indonesia (TSI) Cisarua, Bogor, on the island of Java, from December 2019 to November 2020. The TSI site is located

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at an elevation of 900 to 1800 m above sea level and is located on the north side of Mount Gede. The habitat surrounding the site is comprised of tropical rain forest with interspersed human development and agricultural parcels, and has an average temperature range of 16–24 ° C.

Experimental animals

The Animal Ethics Committee of Insititut Pertanian Bogor (IPB) University approved this research procedure with certificate number 002/KEH/SKE/2020.

The experimental animals used in this study were three Javanese Banteng kept at Taman Safari Indonesia, Cisarua, Bogor (TSI 1). All Banteng originated from Baluran, East Java, and bred at Taman Safari Indonesia 2 (TSI 2) Prigen, Pasuruan, East Java. These three female banteng are of different ages (3, 6 and 10 years old) and are from different genetic bloodlines. Two have calved, one is a heifer, and all are clinically healthy. The three banteng are kept together in one area with separate enclosures at night, that are 4 m x 5 m, but are released in the exhibition area in the morning until late afternoon. Bantengs are fed grass ad-libitum in the exhibition area, 15-20 kg per head in the pens, while they receive concentrate up to 2 kg per head/day. Deworming is performed once every six months and other medications are administered only as needed.

Diagnosis of pregnancy

Generally, pregnancy in bovines is detected by ultrasound on days 11, 18, and 20 after insemination (Rajamahendran *et al*., 1994). The presence of the amniotic sac, heart rate, and embryonic and fetal development are examined. Embryonic and fetal development were measured up to day 60 of gestation.

Monitoring the development of pregnancy

Between day 60 and day 270 following insemination, the development of the placenta is monitored. The length and position of the placenta is measured at the corpus uteri near the cervix. Determination of the gestation period of the three bantengs is based on the results of ultrasound examination at the first detection of pregnancy until birth.

Analysis of the data

Statistical analysis of the obtained data on placental size was performed descriptively and calculated as mean (MEAN) and standard deviation (STDEV).

RESULTS AND DISCUSSION

Ultrasound of a non-pregnant banteng

Histologically, the uterine wall mucosa is a muscular layer composed of myometrium and endometrium, and its surface appears hypoechoic (gray) and compact. Examination of a nonpregnant banteng shows that the uterus is hypoechoic and homogeneous, with a well-defined border with the surrounding tissue and no gestational sacs or white patches suggestive of pregnancy, as shown in Figure 1.

Ultrasound of pregnant banteng

The main objective of early pregnancy diagnosis is to distinguish between non-pregnant and pregnant animals as soon as possible after mating (Torres-Lechuga and Gonzalez-Maldonado, 2022). Non-pregnant banteng has an intact uterus, hypoechoic color, clear demarcation from surrounding issue, and no visible white sacs indicating that the banteng is pregnant (Figure 2). According to Fricke and Lamb (2002), the uterine tissue in non-pregnant and pregnant cows appears as a soft, slightly echogenic, gray structure, while non-pregnant uterus appears compact, and the uterus is more hypoechoic than the surrounding area (Frastantie *et al*., 2019). Diagnosis of Banteng pregnancy began on day 11 after insemination, which was recognized as hypoechoic, homogeneous, and with clear boundaries to surrounding walls. Observation continued on day 18 and was characterized by a 0.27 cm echoless sac with a white patch inside and clear borders to the surrounding hypoechoic tissue (Figure 2). On day 20, the white spot became more prominent, confirming that this was an embryo in an enlarged amniotic sac (Figure 3). The size of the amniotic sac at day 20 was 0.33 cm, which meant that the size increased with gestational age. These results were consistent with those of Fricke and Lamb (2002), who reported that the embryo may be visualized from 20 days after mating and throughout pregnancy.

Imaging results with 5-MHz ultrasound on day 18 after insemination showed a 0.27-cm white bubble with clear borders. According to Adams and Singh (2011), the accuracy of early pregnancy diagnosis before day 18 is not higher than expected (50%). Pregnancy examination by rectal palpation on the 60th day after AI to confirm the ultrasound result on day 18th and ensure that the banteng is pregnant (Setiadi *et al*., 2023). The ultrasound images in Figure 3A show that the chorioallantois gestational sac is anechoic, while the white spots in Figure 3B are growing embryos. These results show that banteng was confirmed pregnant on day 20 after insemination. The corpus luteum was located on the same side as the amniotic sac (ipsilateral) (Figure 2.ii and Figure 3.ii). Kastelic and Ginther (1989) diagnosed pregnancy by ultrasound at days 18-22 with excellent results, but suggested that the investigator must be able to distinguish between chorio-allantoic accumulation in early pregnancy. Scully *et al*. (2014), performed pregnancy diagnosis in the period of days 18 to 21 after insemination in cattle. They suggested that if the cows successfully detected pregnancy, then this was the beginning of the placentation and there was no obvious difference between pregnant and non-pregnant. The early pregnancy ultrasound in cattle differed between operators in their study. The detection accuracy appeared to depend on the ultrasonic device used and the experience of the operator. Cattle embryos can be accurately detected from day 20 of gestation (Adams and Singh 2011). The appropriate time for early diagnosis of pregnancy in dairy cattle depends on the status of the dam—26 days in heifers and the 29 days in cows after insemination (Moharrami *et al*. 2013). In addition, Bekele *et al*. (2016) state that pregnancy tests can be performed in cattle on day 28 after insemination.

In yhidwork, the size of the amniotic sac continued to increase from day 20 and reached 0.33 cm, and the presence of embryos was obvious after the enlargement of the amniotic sac. On day 27 the embryo was shown to be floating as a hypoechoic strucure with a gestational sac of 1.16 cm and there was 0.2 cm of uterine wall thickening. On day 32 of gestation, the chorioallantois bubble enlarged, the embryo was 0.8 cm, and heartbeat and amniotic sac was detectable.

Figure 1. Non-pregnant uterus (A), (B), and (C), hypoechoic in color, clear demarcation from surrounding tissue.

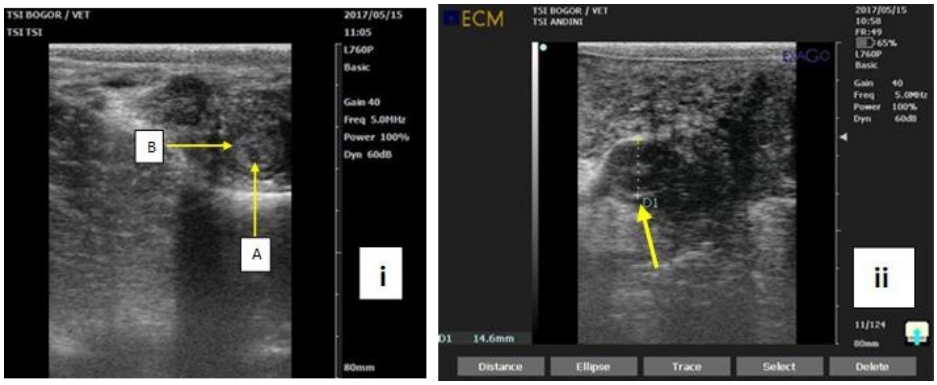

Figure 2. Ultrasound image on day18th after insemination, white sac, white sac (A) and clear demarcation to the surrounding tissue (B)(i), corpus luteum on the same side as gestational sac (arrow) (ii).

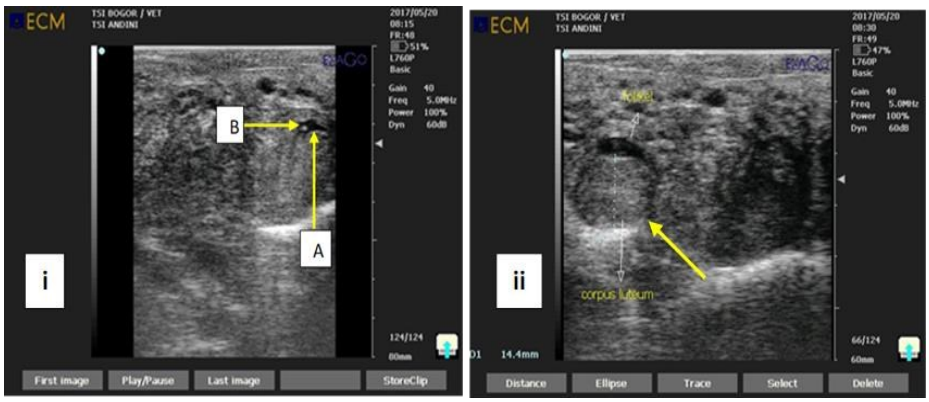

Figure 3. Ultrasound image on day 20 after insemination, the amniotic sac (A) is echoless, and the embryo is hyperechoic within the amniotic sac (B)(i), the corpus luteum is on the same side as the amniotic sac (arrow)

Monitoring of pregnancy

Monitoring of the development of banteng pregnancy was performed on day 27 through day 270 by observing the development of the placenta, and the embryo into a fetus. On day 27 of gestation, the embryo was identified by ultrasound as a 0.55 cm hypoechoic structure with an enlarged 1.16 cm gestational sac. Upon examination on day 32 of gestation, the embryo enlarged to a size of 0.80 cm, and a heartbeat and amniotic sac were noted. On day 40 of gestation, the embryo became larger and measured 1.2 cm, and the embryo was observed more clearly floating in the amniotic sac. When examined on day 53 of gestation, the fetus measured 3.60 cm, and on day 60 it reached a length of 5.04 cm (Figure 4).

Placental development was monitored in all three bantengs from day 60 to day 270 of gestation (Figure 5), and the size of the middle uterine artery (fremitus) was observed every 2–3 weeks following day 150.

The placenta is a major driver of prenatal growth and involved in programming postnatal performance (Burns *et al.* 2018). The placentome was observed and measured in the corpus uteri near the cervix. The results of observation and measurement of the placentome of the three consecutive bantengs are shown in Table 1. The average placental size of the three bantengs from day 60 was 1.21 ± 0.31 cm to day 270 was 5.57 ± 0.26 cm.

The increase in size of the fremitus of the three bantengs showed significant differences until just

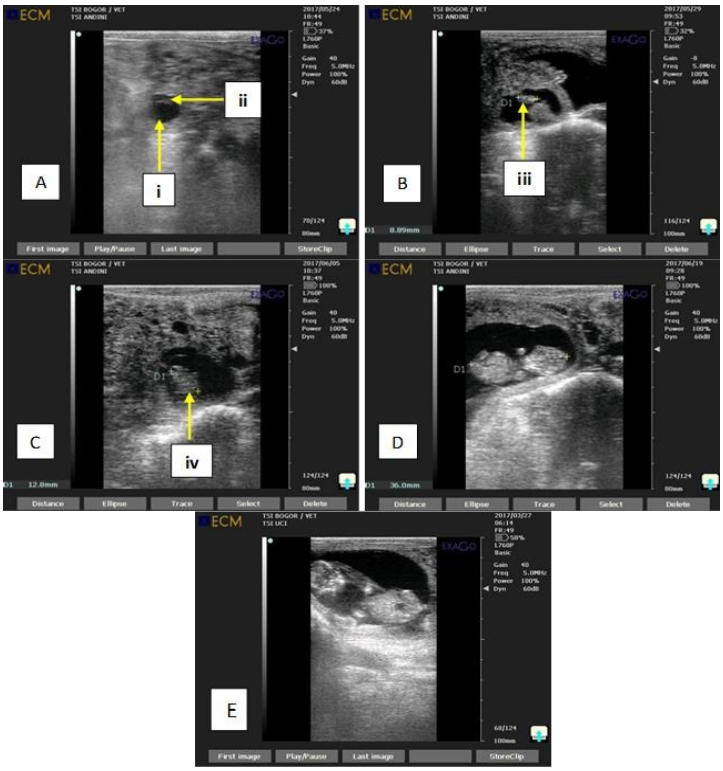

Figure 4. Ultrasound images of the different stages of embryo-to-fetal development in banteng, starting on day 27. They show a floating embryo of 0.55 cm (Ai) and an amniotic sac of 1.16 cm (Aii); on day 32, a CRL embryo of 0.80 cm (Bii); on day 40, a CRL embryo of 1.20 cm (C, arrow); and on day 53, a CRL embryo of 1.20 cm (C, arrow).

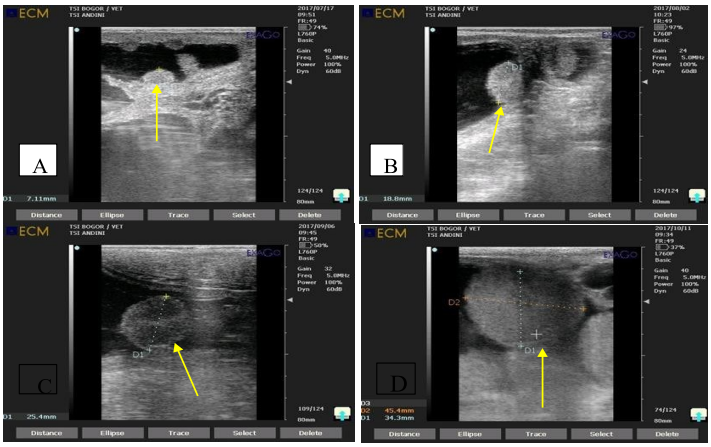

Figure 5. Development of the placentome on day 60 (A), day 90 (B), day 135 (C), and day 180 of gestation.

before birth. The mean size of the fremitus was 0.83 ± 0.14 cm, 1.00 ± 0.23 cm, and 1.17 ± 0.21 cm, respectively.

In bantengs, by day 53 of gestation, organogenesis is complete and the embryo develops into a fetus, with a head to coccyx length (crown rump length/CRL) of 3.6 cm. Clearly visible fetal extremities, tail, and head, and by day 60 of gestation, a fetal CRL of 5.04 cm, visible hyperechoic structures of the os vertebrae and os cervicalis, eyeball, placenta, and sex of the fetus are detectable (Figure 4). Aires *et al*. (2014) state that bovine fetuses at days 50-60 of gestation had a length of 5.0-8.5 cm. The amniotic sac at days 32, 40, 53, and 60 cannot be measured because it has enlarged and widened; it is visible only as a black, anechoic image like a lumen. Measurement of the embryo CRL can only be performed up to day 60 of gestation because the ultrasound probe has limited range or penetration depth to image the object of the enlarging fetus and the uterus, which is almost in a narrow abdominal space and cannot be reached by hand. The gestation period in Banteng ranges from 291 to 299.5 days.

Based on the observations, the placentome on the ultrasound images was identified as an echogenic semicircular shape in the uterine lumen. The size of placentome's on day 60 of gestation ranged from 0.9 cm to 1.52 cm and on day 270 of gestation from 5.26 cm to 5.77 cm. In banteng placentomes from this study, the total size increased from 4.36 cm to 4.25 cm. The placental diameter can be measured by ultrasound on day 35 and day 50 day of gestation and is typically 0.5-0.8 cm (Fricke 2002). At the 7th week of gestation, the

Table 1. Enlargement of placentomes size in pregnant bantengs

Days	Andini	Uchi	Astuti	Means±SD
D60	1.42	1.36	0.85	1.21±0.31
D75	2.02	1.77	0.92	1.57±0.58
D90	2.76	2.41	1.65	2.27±0.57
D105	2.99	2.77	2.94	2.90±0.12
D120	3.89	3.01	3.26	3.39±0.45
D135	4.05	3.28	3.66	3.66±0.39
D150	4.29	3.94	3.99	4.07±0.19
D165	4.47	4.06	3.81	4.11±0.33
D180	4.8	4.6	3.99	4.46±0.42
D195	4.67	4.67	4.07	4.47±0.35
D210	4.9	4.6	4.17	4.56±0.37
D225	5.02	5.01	5.05	5.03±0.35
D240	5.4	5.2	5.4	5.33±0.20
D255	5.7	5.25	5.51	5.49±0.23
D270	5.85	5.35	5.5	5.57±0.26

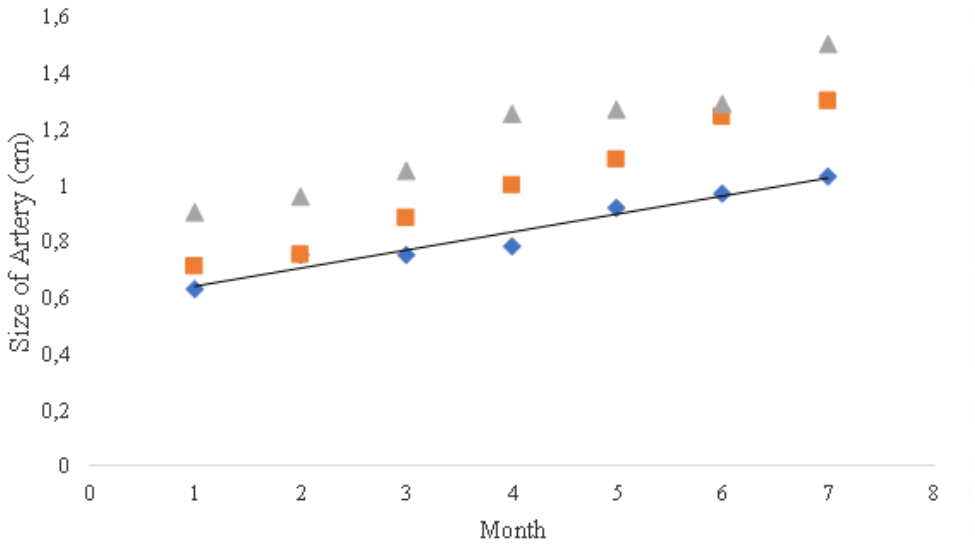

Figure 6. Development of the fremitus of three bantengs from day 150 until shortly before birth.

placental diameter is 7.5 mm and at the 26th week of gestation, it is 69 mm (Rasheed 2018).

Placentomes can be found as early as the 10th week of gestation (Burns *et al.* 2018). The increase in placental size in the first trimester (about 90 days) of pregnancy was 1.69 cm, 1.56 cm in the second trimester, and 1.1 cm in the last trimester. There was a marked increase in size in the first and second trimesters, and in the third trimester, the increase in size slowed down until the 270th day of gestation. In *Bos taurus-Bos indicus*, the number of cotyledons in the expelled placenta was increased by a high protein content in the diet during the second trimester of pregnancy and varied according to genotype (Burns *et al.* 2018). The continuity of pregnancy progression is maintained by the hormone progesterone, which is derived primarily from the corpus luteum in addition to the placenta. although the corpus luteum is the main source of P4 for the maintenance of pregnancy.

CONCLUSION

Early pregnancy in banteng can be detected on day 20 after mating by the presence of a pregnant amniotic sac (chorioallantois) and floating white spots. The development of placental size during pregnancy can be assessed by ultrasound.

CONFLICT OF INTEREST

The researcher declares that there are no financial, personal, or other conflicts of interest with any other person or organization related to the material discussed in this article.

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