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The accuracy of progesterone test kits for early diagnosis in Ettawa cross-goat

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Abstract

This research aimed to determine the accuracy of the progesterone test kit for milk and blood for early pregnancy diagnosis in Ettawa cross goats (PE). Five lactating PE goats were used in this study and were synchronized with PGF2α intramuscularly. The result is that all goats showed a sign of estrus. Four goats were mated naturally, while one other was not mated. The determination of day 0 was when the females were standing heat. The early pregnancy diagnosis using dairy cow test strips was performed 18-22 days after mating or post-estrus. The accuracy of the diagnosis was confirmed by ultrasound on day 35 after mating/post estrus. Diagnosis using the milk progesterone kit showed negative results for five days of examination in all goats, whereas using the blood progesterone kit showed positive results in four goats. When confirmed, the ultrasound showed that one goat was not pregnant, and four other goats were pregnant. It can be concluded that the pregnancy test strip kits containing progesterone are effective in diagnosing early pregnancy in PE goats with high accuracy in blood samples (100%) despite low accuracy in milk samples (20%).

Keywords: early pregnancy diagnosis, PE goat, progesterone kits.

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Background

A critical aspect of reproductive management is early pregnancy detection. Early pregnancy detection will provide information about mating success more quickly, so further treatment can be carried out immediately (Karen et al., 2004). It can reduce the risk of abortion, stillbirth, or birth defects and optimize medical treatments and feed costs (Wani et al., 1998). Therefore, there is a need for an early pregnancy diagnosis method that is easy to perform, safe for both breeders and examiners/owners, inexpensive, and has high accuracy (Siregar & Hamdan, 2008).

Various pregnancy detection methods that currently exist, especially in dairy goats, include observing the reappearance of heat after mating (non-return to the estrous method) (Syafruddin et al., 2012), abdominal palpation (Hafez, 2000), ultrasound (Melia et al., 2018), analysis of pregnancy-associated proteins (Karadaev, 2015), and measurement of progesterone levels in blood and milk using a kit (Karadaev, 2015).

Until now, the method for detecting pregnancy often used by breeders is the non-return to the estrous method (observation of the reappearance of heat after mating). The accuracy of this method for diagnosing pregnant and not pregnant in dairy goats was 93.75 and 37.5%, respectively (Syafruddin et al., 2012). According to Siregar and Hamdan (2008), there are several areas for improvement in the non-return to the estrous method. Some cattle do not show estrus symptoms even though they are in estrus. This relates to silent estrous or sub-estrus. This number increases during heat stress, especially in cattle with reproductive disorders such as ovarian cysts, uterine infection, or anestrus. They can fail to show estrus and are diagnosed as pregnant. Some pregnant herds show symptoms of heat, although this is rare, and livestock with abnormal cycle lengths (>25 days) or early

embryonic death will result in an erroneous diagnosis.

In addition to the basic techniques, early pregnancy conclusion can be recognized by utilizing hormonal examination methods and test units for the conclusion of pregnancy. One of these strategies is pregnancy testing by measuring progesterone levels in blood and breast milk. Pile et al. (1973) reported that the concentrations of progesterone in blood and milk at 20-24 days post-insemination were examined to analyze pregnancy in cattle.

The measurement of progesterone levels for pregnancy diagnosis is generally divided into quantitative and qualitative methods. Quantitative methods can determine the progesterone's absolute concentration, such as radio immune assay (RIA) and enzyme-linked immunosorbent assay (ELISA) techniques. In the mid-1980s, kits for performing the progesterone detection procedure using the ELISA method and agglutination tests in milk were available for dairy farmers (Broaddus & De Vries, 2005). Qualitative methods can only display the relative progesterone concentration and produce a color or agglutination reaction. The qualitative method of examining progesterone in milk is considered the on-farm milk progesterone test or cow side test because it can be carried out in a stable or veterinary clinic, and the results can be seen within 5-10 minutes (Nebel, 1998; O'Connor, 2003).

Bretzlaff et al. (1989) reported that the milk progesterone enzyme immunoassay (EIA) kit is more widely used to diagnose early pregnancy in cattle. Dionysius (1991) reported that using a milk progesterone kit to diagnose pregnancy in goats on the 21st and 24th day after mating had an accuracy rate of 83-88%. Another study by Engeland et al. (1997) also reported that the accuracy rate of using breast milk progesterone on the 20th day was 82%. In addition, progesterone levels can be measured using blood samples, especially serum (Fleming et al., 1990).

Nova et al. (2014) reported that the use of the one-step dairy cow pregnancy test cassette® (milk) milk progesterone kit performed on PE goats on days 18-22 after mating had a high accuracy rate of 80% for the diagnosis of non-pregnancy. The results of this study put the breast milk progesterone kit method as the fastest procedure to identify non-pregnant female animals.

Of the several commercial kits on the market, one of the kits that can diagnose early pregnancy is the dairy cow pregnancy test strip® (milk or blood) (Span Biotech Limited) used on dairy cows. However, no research has reported using this kit to diagnose pregnancy in Ettawa cross (PE) goats.

Materials and Methods

This study used five PE female goats aged 3-4 years in the lactation period and weighing 30-35 kg. A male PE goat aged 3.5 years weighing 45 kg was used as a stud. Goats were placed in a group and numbered (1-5). The goat No. 1 was not mated, while the goats No. 2-5 were mated. All PE female goats were synchronized using 1 ml of prostaglandin 2α (PGF2α) intramuscular injection. The males were then brought closer to the females in their cages to initiate symptoms of heat faster. Arousal observations were conducted in the morning, afternoon, and evening.

Four PE goats showing symptoms of heat were bred naturally, while the other one showing the same symptoms was not mated. The determination of day 0 in this study was when the PE female goat was at the peak of estrus (not resisting when being ridden).

Diagnoses of pregnancy using the dairy cow pregnancy test strip® (milk or blood) were carried out 18-22 days after mating or 18-22 days after estrus in non-mated goats. The goats' pregnancy status was confirmed on day 35 after mating and after estrus using ultrasonography (USG).

Pregnancy Check using a Progesterone Kit

The use of the test kit followed the instructions listed in the product protocol. The milk and blood samples were put into 5-10 ml different sample bottles/measuring cups. Before being collected, the milk from the first to the third collection was discarded, while the fourth to the next milk was collected in a sample bottle or

measuring cup. The blood was drawn from the jugular vein. The test strips were then dipped in milk and blood using different test strips. The results were read after 5 minutes. Positive pregnancy results showed red on the test line (T) and control line (C), while negative pregnancy results showed red on the control line (C) only. The sample was invalid if the control and test lines showed no color change.

Procedure for Using Transcutaneous Ultrasound

The ultrasound device (MINDRAY DP3300 VET, Shenzhen Mindray Bio-Medical Electronic Co. Ltd, China) was placed to the left of the operator's arm in the goat's left position. Scanning was performed with a 3.5 Mhz abdominal probe (35C50EB, Shenzhen Mindray Bio-Medical Electronic Co. Ltd, China) while the female goat was lying down. Part of the hair was shaved in order to show the best image visualization. A lubricant such as KY jelly was applied around the abdomen, and then the probe was directed from the cranial mammary gland to the sebum that filled the inguinal gland, then directed dorsally and slightly caudomedial. The probe was slightly pressed on the abdomen towards the urinary bladder. A gestational sac and an embryo/fetus indicate that the goat is pregnant (Melia et al., 2018).

Data Analysis

The parameter of this study was the number of female goats diagnosed positively and negatively pregnant in each treatment group using the milk and blood progesterone kit at 18-22 days after mating and then confirmed using ultrasound on the 35th day after the mating. Data regarding the number of female goats diagnosed as pregnant or not pregnant using the milk and blood progesterone kit in treated goats were analyzed descriptively.

Results and Discussion

In the early pregnancy examination using a progesterone kit for milk and blood, five PE goats were used. Four PE goats underwent heat synchronization and then mating, while the other one got synchronized but not mating. Collection and examination of milk and blood samples begin 18-22 days after mating and after estrus. Samples were taken every 07:30 AM for five consecutive days. The results of a pregnancy diagnosis test using a progesterone kit in PE goats with milk and blood samples are presented in Figure 1 and Table 1.

According to Nebel (1998), progesterone examination in milk and blood samples can be read within 5-10 minutes. However, the results showed a different reaction time than the progesterone kit used. The estimated time needed to examine milk and blood samples is 30-40 minutes. The different result of the reaction in this particular kit is suspected because the sample used has a high viscosity, which may take quite a long time to disperse on the test kit compared to the time stated on the product brochure.

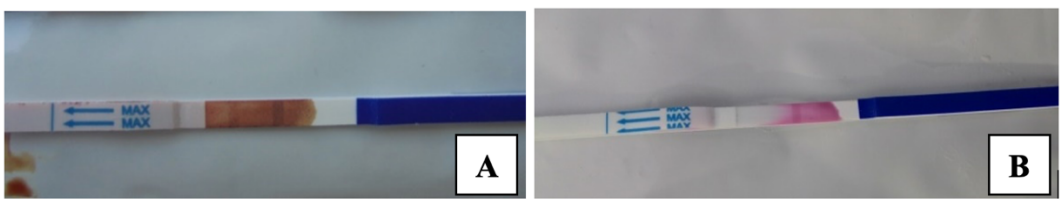

Figure 1. Pregnancy test results with a blood progesterone kit showed positive results (A), and pregnancy testing results with a breast milk progesterone kit showed negative results (B).

Table 1. The results of the PE goat's need for a diagnosis using the progesterone kit on milk and blood samples

No. goats	Pregnancy diagnosis results		
	Progesterone kit (Milk)	Progesterone kit (blood)	Confirm using USG
1^K	-	-	-
2	-	+	+
3	-	+	+
4	-	+	+
5	-	+	+
Accuracy (%)	20%	100%	

1^K : Goat PE control (Not Mated)
(-) = negative pregnant
(+) = positive pregnant

Table 1 shows that the results of examining the hormone progesterone using the progesterone kit on the milk samples showed negative results for all experimental goats. After being confirmed using ultrasound on the 35th post-mating day, four PE goats were diagnosed positive pregnant, and the other one was diagnosed negative (goat No. 1), so it can be stated that the accuracy of the progesterone kit in milk samples for the diagnosis of pregnancy and not pregnancy is 0% and 20%. The results of this examination follow the research reports of Lestari (2006) and Nova et al. (2014), which state that using a breast-progesterone kit is more accurate for diagnosing non-pregnant status than pregnant status. This can help identify non-pregnant goats much more quickly than the non-return to the estrous method.

The low accuracy of the progesterone kit in breast milk samples for the diagnosis of pregnancy is caused by several factors. According to Maryati and Nuniek (1990), progesterone levels in milk are affected by the feed and fat content in the milk. According to Holdsworth and Davis, another factor that causes the level of progesterone in breast milk is not always constant (Nova et al., 2014). Pregnanedione compound is formed from the metabolism results of udder cells against progesterone in milk. Therefore, the progesterone concentration in breast milk often fluctuates on each examination day.

The data shown in Table 1 for the results of a pregnancy diagnosis using a progesterone kit on a blood sample shows a different diagnosis result from a breast milk sample. In the blood sample, four PE goats were diagnosed positively pregnant, namely PE goats No. 2, 3, 4, and 5, while PE goat No. 1 was diagnosed negatively pregnant. After being confirmed using ultrasound on the 35th day after mating, the results were the same as those of tests using blood samples. There were four PE goats diagnosed as positively pregnant and one PE goat diagnosed as negatively pregnant, so it can be stated that the accuracy of the progesterone kit in blood samples for the diagnosis of pregnancy and not pregnancy is 100%, respectively.

The accuracy of the progesterone kit with blood samples in this study was higher than that reported by Boscos et al. (2003), who used the EIA method to diagnose goat pregnancy and then confirmed it at delivery, which had an accuracy of 71.5% for determining pregnancy status. The same result was also presented by Islam et al. (2014) using the ELISA method to diagnose the pregnancy status of Black Bengal goats, which has high accuracy for detecting pregnancy 25-30 days post mating.

An overview of the results of the ultrasound examination of experimental goats diagnosed as pregnant and not pregnant is presented in Figure 2.

From the results of ultrasound observations in Figure 2A, it is clear that the amniotic fluid is black (anechoic), and the embryo is gray. In Figure 2B, no embryos and amniotic fluid are visible; it can be assumed that the goat is not pregnant. According to Amer (2008), the diagnosis of female goats' pregnancy status using ultrasound is if there is an embryonic vesicle filled with anechoic fluid (black) and cotyledons and parts of the fetus are visible.

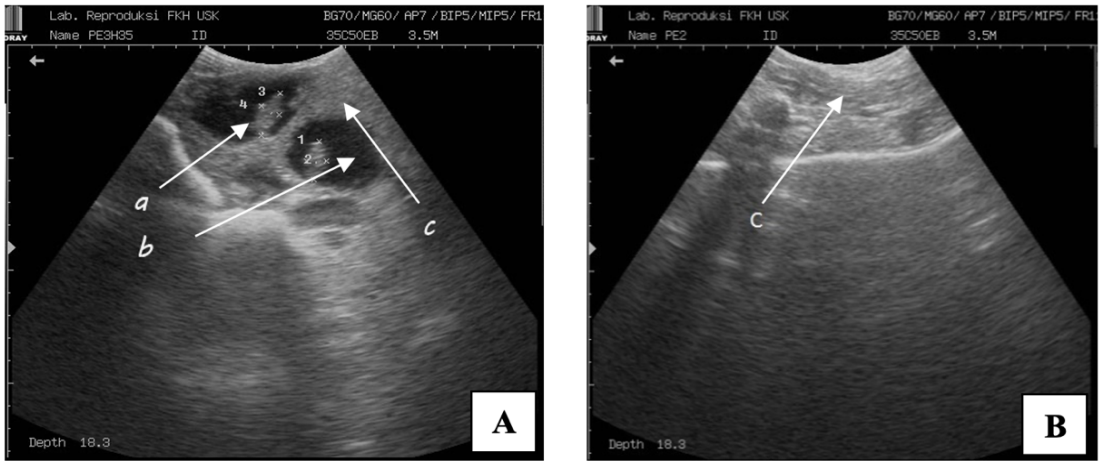

Figure 2. Ultrasound images of PE goats diagnosed with pregnancy on day 35 post-mating (A) and ultrasound images of goats diagnosed as not pregnant on day 35 post-estrus (B). (a=embryo, b=amnion, and c=uterus).

Conclusion

A kit of progesterone pregnancy test strips (milk or blood) used in dairy cows can effectively diagnose early pregnancy in PE goats using blood samples. This progesterone kit has high accuracy in blood samples (100%) and low accuracy in breast milk samples (20%).

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