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 as means of 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 sigh 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. Early pregnancy diagnosis using dairy cow test strips performed 18-22 days after mating or post-estrus. The accuracy of the diagnosis was confirmed by ultrasound examination 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, using ultrasound showed one goat was diagnosed as not pregnant, and four other goats were diagnosed pregnant. It can be concluded that the use of progesterone dairy cow pregnancy test strip kits is effectively used to diagnose early pregnancy in PE goats with high accuracy in blood samples (100%) and low accuracy in milk samples (20%).

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

Background
One important aspect of reproductive management is early pregnancy detection. Early pregnancy detection will provide information about mating success more quickly so that failure evaluations can be carried out immediately (Karen et al., 2004), such as reducing the risk of abortion, stillbirth or birth defects and optimizing medicine 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 most often used by breeders is the non-return to the estrous method (observation of 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, cattle with reproductive disorders such as ovarian cysts, uterine infection, or anestrus also 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 over, early pregnancy conclusion can presently be recognized 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 blood and milk concentrations of progesterone at 20-24 days post-insemination had been utilized to analyze pregnancy in cattle.

Measurement of progesterone levels for the diagnosis of pregnancy is generally divided into two methods, quantitative and qualitative. Quantitative methods can determine 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 colour or agglutination reaction. The qualitative method of examining progesterone in milk is termed 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).

Bretzloff 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. Until now, no research report has reported using this kit to diagnose pregnancy in Ettawa cross (PE) goats.

Materials and Methods

In this study, five PE female goats were used during lactation, aged 3-4 years and weighing 30-35 kg. A male PE goat aged 3.5 years weighing 45 kg was used as a stud. Goats are placed in a group and numbered (1-5). PE goats No. 1 are goats that are not mated, while PE goats numbers 2-5 are mated. All PE female goats were synchronized using PGF2α and injected intramuscularly as much as 1 ml. To speed up the symptoms of heat, the males are brought closer to the females, and the males are kept side by side with the female's cages. Aroused observations were made in the morning, afternoon and evening.

A total of four PE goats showing symptoms of heat were bred naturally, while one other goat showing symptoms of heat 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).

Diagnosis of pregnancy using the kit progesterone milk and blood dairy cow pregnancy test strip® (milk or blood) is carried out at 18-22 days after mating or at 18-22 days after estrus in non-mated goats. Confirmation of the pregnancy status of the experimental goats was carried out on the 35th day after mating and after estrus using ultrasonography (USG).

Pregnancy Check using a Progesterone Kit

The use of the test kit is following the instructions for use listed in the product protocol. Milk and blood were put into 5-10 ml different sample bottles/measuring cups. Before being collected, the milk from the first to the third is discarded first, while the fourth to the next milk is collected in a sample bottle or measuring cup. The blood sample comes from the jugular vein. The test strip is dipped in milk and blood using different test strips. Results were read after 5 minutes. Positive pregnancy results show red on the test line (T) and control line (C),
while negative pregnant results show red on the control line (C) only. The sample is said to be invalid if the control line and test line show 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 mother goat was lying down. Part of the hair was shaved before the evaluation was carried out in order to show the best image visualization, KY jelly was applied around the abdomen in front of the mammary gland, and then the probe was directed to the cranial mammary gland to the sebum that filled the inguinal gland, then directed dorsally and slightly caudomedial. The probe is slightly pressed on the abdomen towards the urinary bladder. The presence of a gestational sac and the presence of an embryo/fetus indicates that the goat is pregnant (Melia et al., 2018).

**Data Analysis**

The research parameter 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, namely four PE goats were synchronized with heat and then mated, and one PE goat was synchronized with heat but not mated. Collection and examination of milk and blood samples begin 18-22 days after mating and after estrus. Samples were taken every 07:30 WIB 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.

![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).](image)

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

<table>
<thead>
<tr>
<th>No. goats</th>
<th>Pregnancy diagnosis results</th>
<th>Confirm using USG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Progesterone kit (Milk)</td>
<td>Progesterone kit (blood)</td>
</tr>
<tr>
<td>1^K</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Accuracy (%)**

| Accuracy (%) | 20% | 100% |

1^K: Goat PE kontrol (Not Mated)

According to Nebel (1998), progesterone examination in milk and blood samples can be read within 5-10 minutes, but data on goats numbered 1-5 samples show a different reaction time than the progesterone kit used. The estimated time needed to examine milk and blood samples is 30-40 minutes. The result of the reaction in a kit like this is suspected because the sample used has a high viscosity. This causes the sample, when dropped on the progesterone kit to move slowly and takes
quite a long time compared to the time stated on the product brochure.

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 positively pregnant, and one PE goat was diagnosed negatively pregnant (goat No. 1), so it can be said 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 states 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), hormone 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. (1979) in Nova et al. (2014) is a pregnanedione compound. This 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 said 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, 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 has high accuracy for detecting pregnancy which is examined 25-30 days after mating.

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

Figure 2. Ultrasound image of PE goats diagnosed with pregnancy on the 35th day post-mating (A) and ultrasound images of goats diagnosed not pregnant on the 35th day post-estrus (B). (a=embryo, b=amnion, and c=uterus).
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; this can be assumed that the goat is not pregnant. According to Amer (2008), the diagnosis of female goats’ pregnancy status is ultrasound if there is an embryonic vesicle filled with anechoic fluid (black) and cotyledons and parts of the fetus are visible.

Conclusion

A kit of progesterone dairy cow pregnancy test strips (milk or blood) in dairy cows is effectively used to 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%).

References


