Research Article

Comparison of Pregnant Diagnosis in Local Buffalo with a Seed Germination Inhibition Test and Rectal Palpation

Ferry Lismanto Syaiful[1*], Jaswandi Jaswandi[2], Mangku Mundana[2], Ilham Ilham[2], Novirman Jamarun[1], Efrizal Efrizal[3]

[1]Faculty of Animal Science, Andalas University, Padang, West Sumatra, Indonesia; [2]Faculty of Animal Science, Andalas University, Padang, West Sumatra, Indonesia; [3]Department of Biology, Faculty of Mathematics and Natural Science, Andalas University, Padang, West Sumatera, Indonesia.

Abstract | Pregnancy detection is the most essential aspect after insemination, this can increase reproductive efficiency and buffalo birth. This study is aimed is evaluating the use of mung bean seeds on multiple dosages in the growing seeds tests for the accuracy, sensitivity, and phases of local buffalo pregnancy, and detecting buffalo pregnancy quickly, massively, and cheaper. The object of this study was 40 local buffalo post-AI with the criteria of BCS ≥3 and 2-3 parturition. The research medium used was green bean seeds. Then use urine and water samples. The urine samples used in this study were 120 samples. The dosage uses the ratio of mixing urine and water 1:12 mL, 1:14 mL, and 1:16 mL. The variables of this research are accuracy, sensitivity, and the phases of local buffalo pregnancy. This research shows that the level of local buffalo on the days-21; 42; 63 after AI is 7.50%; 55%; and 70%. The accuracy of local buffalo pregnancy is 78.57%. Moreover, the sensitivity of buffalo pregnancy using mung bean seed in multiple dosages of treatment (1:12; 1:14; 1:16mL) on days-21; 42; 63 after AI shows results of 10.71%; 78.57%; 100%. The finding of this research shows that the use of mung bean seed can detect the phases of local buffalo pregnancy with up to 70% accuracy; the sensitivity of buffalo pregnancy is 78.57% and 100% pregnancy accuracy. Buffalo pregnancy test using a seed germination inhibition test can detect pregnancy easily, practically, massively, and more economically.

Keywords | Accuracy, Sensitivity, Local buffalo pregnancy, Seed germination inhibition, Rectal palpation, Bio-assay test

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***Correspondence** | Ferry Lismanto Syaiful, Engineer Professional Program, Faculty of Animal Science, Andalas University, Padang, West Sumatra, Indonesia; **Email:** ferrylismanto@ansci.unand.ac.id, ferrylismanto5@gmail.com
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INTRODUCTION

Buffalo is a multi-purpose livestock that produces meat, milk, labor, etc. Buffaloes have several advantages, including taking advantage of low-quality feed, high adaptation, disease resistance, and docile temperament. However, buffalo productivity must improve due to long spacing between calves and low birth rates. Buffalo's productivity must be increased through artificial insemination (AI). AI is effective and efficient in improving the genetic quality of livestock. According to Syaiful et al. (2019), the success of artificial insemination (AI) can increase reproductive efficiency and childbirth.

Early detection of buffalo pregnancy is essential after insemination (AI) to determine the success rate of artificial insemination. According to Van Vleck Pereira et al. (2013), Syaiful et al. (2017, 2019), and Syaiful (2018), early pregnancy detection is the most important step taken to identify post-insemination pregnancy. If the cattle are not pregnant, they can repeat the insemination; otherwise, if the cattle are pregnant, they can take good care of the livestock. This process helps lower production costs and can increase childbirths. Reinforced by Sayuti et al. (2011), the accuracy of early pregnancy pregnancy tests can detect embryo death to reduce high production costs.

According to Paws and Purohit (2013), Das et al. (2011), Wani et al. (2003), cattle pregnancy detection techniques have been carried out including, rectal palpation, hormonal tests, and ultrasound (ultrasonography) (Paws and Purohit, 2013; Ramoun et al., 2019), urine metabolic analysis (Sarangi et al., 2022), ELISA (enzyme linked-immunosorbent assay) (Tadeo, 2021; Arshad et al., 2022), progesterone test (Wani et al., 2003). Pregnancy detection technology has advantages and disadvantages, such as practical application, simplicity, accuracy, cost, and non-invasive techniques. Ramoun et al. (2019) suggested that pregnancy detection technology can increase livestock productivity.

According to Sianangama et al. (2022), Bethapudi et al. (2015), Veena (1997), a seed germination inhibition test is a technology for diagnosing livestock pregnancy using simple, non-invasive techniques. Application of technology is more effortless, massive, inexpensive, and does not require special skills. Research on a seed germination inhibition test has been carried out, including tests of sorghum seed germination on buffalo (Rahman and Saha, 2020), rice seed germination tests, wheat and corn seed germination test in cattle, and Pesisir cattle (Syaiful et al., 2017; Syaiful, 2018); seed germination test on Malnad Gidda cattle (Swamy et al., 2010); seed germination test on buffalo (Aswathnarayanappa et al., 2019; Hussain et al., 2016; Skalova et al., 2017).

This method is an alternative for detecting pregnancy in livestock, which is more affordable. However, there has been no research on a seed germination inhibition test using mung bean seeds that reports it is related to local buffalo. This study aims to determine the effectiveness of using mung beans in a seed germination inhibition test on local buffalo's accuracy, sensitivity, and pregnancy rate. The application of this technology is expected to detect pregnancy in local buffalo easily, practically, massively, and economically.

MATERIALS AND METHODS

This study used 40 (forty) buffalo after artificial insemination (AI). The criteria for the research buffalo were BCS ≥3, not pregnant, parity 2-3. The materials used in this study were buffalo urine, distilled water, and green beans. The equipment used is a petri dish, measuring cup, tweezers, urine bag.

Collection of urine

The urine samples used were 40 local buffalo urine after AI. Urine samples were taken in the morning. According to Rine et al. (2004), Boudra et al. (2022), urine is collected by urinary induction, which is done by stroking the thick skin just below the vulva continuously. The urine obtained is then filtered to make it clean, sterilized in a dry plastic container, and then put in the urine to be taken to the laboratory. This urine collection was carried out on days 21, 42, and 63 after IB.

Procedure: A seed germination inhibition test

The procedure for a germination inhibition test refers to the standard procedure Veena (2006) with several modifications. As many as 15 seeds of treated green beans were used and then put in a petri dish that had been given filter paper. Furthermore, 15 mL of urine is diluted with water in a 1:12, 1:14, 1:16mL ratio. Petri dishes filled with seeds using 15mL of water as a control.

The treated mung bean culture was stored in the laboratory for five days at around 25^{o}C. Observations of green bean seed germination were observed from day-1 to day-5; if, after five days, the growth of the shoots decreased/did not grow, then it was considered pregnant (positive).

Pregnancy detection of local buffalo using a seed germination inhibition test was carried out on days-21, 42, and 63 after AI. The buffalo pregnancy was detected using rectal palpation on the 90th-day. The variables of this study were accuracy, sensitivity, and pregnancy rate of local buffalo. The calculation of the accuracy of the buffalo pregnancy is sensitivity, specificity, positive and negative predictive value (Pieterse et al., 1990). The data obtained were analyzed using SPSS 23.0 based on the chi-square test.

Percentage of a seed germination inhibition (SGI) by calculating the formula:

$$SGI\ \% = \frac{\text{Total of a seeds not germination in Petri dish}}{\text{Total of a seeds not germination taken in Petri dish}} \times 100\%$$

Table 1: Calculation formula sensitivity, specificity, and overall diagnostic accuracy in local buffalo.

No	Pregnant	Non-Pregnant
1.	Diagnosis pregnant correct (a)	Diagnosis non-pregnant correct (c)
2.	Diagnosis pregnant incorrect (b)	Diagnosis non-pregnant incorrect (d)

Total pregnant buffaloes = a+d
Total buffaloes non-pregnant= b+c
Sensitivity (Sp;%) = a/(a+d) x 100%
Specificity (Sp;%) = c/(c+d) x 100%

Positive predictive value (PPV;%) = a/(c+d) x 100%
Negatif predictive value (PPV;%) = c/(c+d) x 100%
Overall diagnostic accuracy = a+c/ (a+b+c+d) x 100

RESULTS AND DISCUSSION

Pregnancy rate of local buffaloes

The pregnancy rate of local buffalo using a seed germination inhibition is shown in Figure 1.

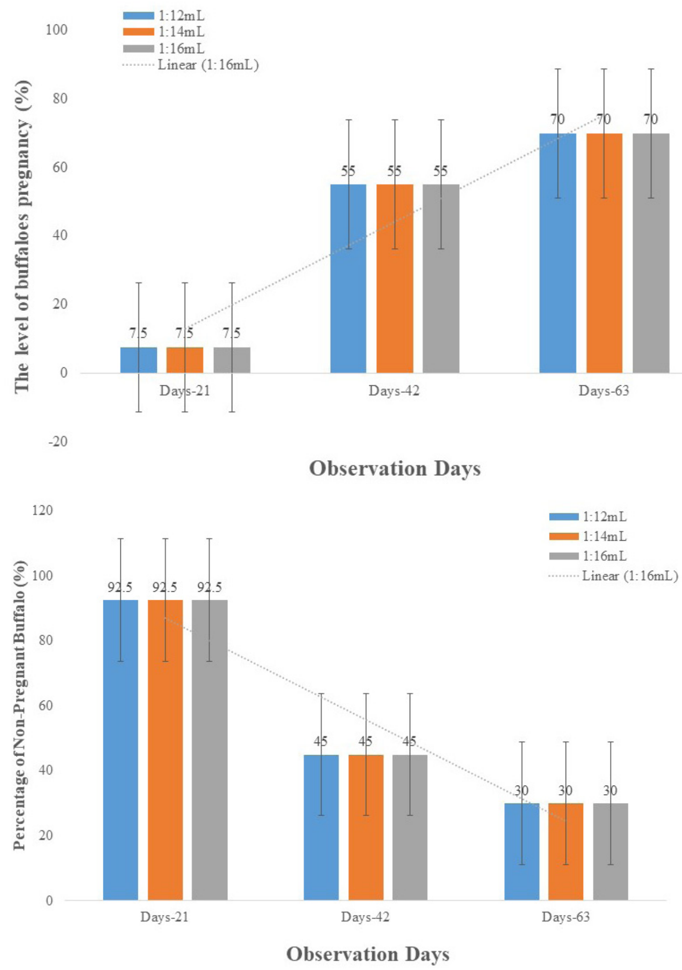

Figure 1: Buffalo pregnancy rate in the a seed germination inhibition method using green beans (%).

Figure 1 shows that the pregnancy rate of buffalo using mung beans in the seed germination test method significantly differs from the day of observation. The older the gestational age, the higher the buffalo's pregnancy rate, and vice versa. His difference is due to an increase in the hormone abscisic acid (ABA) level in the urine, thereby inhibiting the growth of green bean seeds. Veena (2006) states that pregnant cattle urine contains the hormone ABA (Abscisic acid); the higher the gestational age, the higher the ABA hormone levels in the urine. The urine of pregnant cows had a higher abscisic acid concentration of 170.62 nm/ml, while the urine of non-pregnant cows only had an abscisic acid concentration of 74.46 nm/ml. Hussain and Muneebullah (2016) state that increasing the concentration of ABA can extend the dormant period of seeds soaked in water. This is an indicator for detecting pregnancy in seed germination inhibition.

The seed germination inhibition test technique can detect cattle pregnancy through urine. The results of this study follow Aswathnarayanappa et al. (2019), Dilrukshi dan Perera (2012), Hussain and Muneebullah (2016), Perumal (2014), Swamy et al. (2010), Krishna dan Veena (2009), Dilrukshi and Perera (2009), that seed germination inhibition can detect livestock pregnancy. Dilrukshi and Perera (2009) stated that there is no difference in ABA concentrations in cattle that are not pregnant. ABA is a plant hormone that can inhibit seed dormancy; differences in ABA were detected in the urine of pregnant and non-pregnant cattle at different concentrations. Sianangama et al. (2022), the higher percentage rate of seed germination inhibition may be due to ovarian steroids and other substances in the urine of pregnant cows. The difference in dose (urine: water) was not significantly different from the pregnancy rate of the buffalo. However, the older the gestational age, the more likely the seeds do not grow. According to Purwaningsih (2001), Syaiful et al. (2017), Syaiful (2018), Laznickova et al. (2020), gestational age can inhibit seed germination caused by the ABA hormone. ABA will inhibit the growth of seed sprouts by inhibiting nucleic acid synthesis and protein synthesis, thereby affecting the process of sprout growth. Using green beans in the seed germination test method can detect post-insemination buffalo pregnancy. Higher buffalo pregnancy rates were obtained on the 63rd day by 70%.

Accuracy of pregnancy detection in local buffalo

The pregnancy results of buffaloes using different pregnancy detection methods are shown in Table 2.

Table 2: Comparison of the pregnancy diagnosis in buffalo using the A seed germination inhibition test and rectal palpation (n=40).

No	Result of diagnosis	Observation days	A seed germination inhibition test (%)	Rectal palpation (%)	Diagnosis incorrect (%)
1.	Pregnant	Days-21	7.5	70.0	30.0
		Days-42	55.0	70.0	30.0
		Days-63	70.0	70.0	30.0
2.	Non pregnant	Days-21	92.5	30.0	70.0
		Days-42	45.0	30.0	70.0
		Days-63	30.0	30.0	70.0

The results of this study are related to the percentage of seed germination inhibition. Table 2 shows that the highest

Table 3: Pregnancy detection accuracy using a seed germination inhibition test (n=40).

No	Particulars of buffaloes tested		Total pregnancy detection of buffaloes (%)
1.	Diagnosis pregnant correct	:	70.0
2.	Diagnosis non-pregnant incorrect	:	30.0
3.	Diagnosis non-pregnant correct	:	30.0
4.	Diagnosis non-pregnant incorrect	:	70.0
5.	Sensitivity (Sp;%) = a/(a+d) x 100%	:	50.0
6.	Specificity (Sp;%) = c/(c+d) x 100%	:	30.0
7.	Positive predictive value (PPV;%) = a/(c+d) x 100%	:	70.0
8.	Negatif predictive value (NPV;%) = c/(c+d) x 100%	:	30.0
9.	Overall diagnostic accuracy = a+c/ (a+b+c+d) x 100	:	50.0

buffalo pregnancy rate was obtained on the 63rd day (70%) and the lowest on the 21st day (7.5%). In contrast, in non-pregnant cattle, the highest was obtained on the 21st day (92.5%), and the lowest was on the 63rd day (30%). The pregnancy rates for pregnant/ non-pregnant buffaloes and observations on the day of pregnancy diagnosis statistically showed a significant difference ($p<0.05$). Rai et al. (2018); Dilrukshi and Perera (2009) found that the urine of pregnant cattle has more ABA levels than non-pregnant cattle.

According to Dilrukshi and Perera (2012), Hussain and Muneebullah (2016), seed germination can be hampered due to increased levels of the hormone abscisic acid (ABA). ABA can also lower the urine pH of pregnant cattle, which causes an increase in ABA levels so that it can increase plant dormancy. On the other hand, the urine of pregnant cattle also contains the hormones estrogen and progesterone. However, according to Nirmala et al. (2008), estrogen and progesterone did not affect seed germination and shoot length.

Accuracy of pregnancy detection in local buffalo using a seed germination inhibition test shown in Table 3.

Table 3 shows that using the seed germination inhibition test obtained a positive predictive value (PPV) of 70% and NPV (30%). This shows that the urine of pregnant cattle significantly suppressed seed germination. The results of this study are higher than Dilruksi and Perera (2009); using the seed germination test resulted in a cow's pregnancy rate (57.93%). This difference is caused by the type of livestock, seed, dose, and media used.

The use of seed germination inhibition and rectal palpation did not differ in the pregnancy rate of cattle by 70% vs. 70%, Sensitivity (50%), Specificity (30%), and overall diagnostic accuracy of 50% (Table 3). Seed germination inhibition can detect pregnancy on the 21st and 42nd day after AI. However, these two tests have limitations due to pregnant and non-pregnant cattle's high overall diagnostic accuracy. Krishna and Veena (2009), the superiority of the seed germination inhibition method can detect pregnancy early. Added Bethapudi et al. (2015), this pregnancy detection method is inexpensive and does not require special skills.

Farmers can detect pregnancy in buffalo using seed germination inhibition because this method can be done quickly, is inexpensive, non-invasive, and is more efficient in detecting pregnancy in cattle.

CONCLUSIONS AND RECOMMENDATIONS

Pregnancy detection is essential in reproductive management, which helps increase reproductive efficiency and calf birth. The study results showed that the seed germination inhibition test could detect cattle pregnancy with a buffalo pregnancy rate of up to 70%, sensitivity (50%), specificity (30%), and overall diagnostic accuracy of 50%.

Using a seed germination inhibition technique is reliable in detecting pregnancy in cattle. This technique is easier and more economical and can be applied in the field. However, the rectal palpation method requires experience, special expertise, and knowledge of the buffalo reproductive system. Based on the above, it can be concluded that pregnancy detection using the seed germination inhibition test is suitable for farmers to detect pregnancy in their livestock.

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NOVELTY STATEMENT

This research aims to assess the accuracy, sensitivity, and specificity in detecting pregnancy in local buffalo using different methods (such as the green bean seed germination test and rectal palpation). One key aspect of this study involves employing local buffalo urine and green bean seeds as the growth medium, administered in various treatment doses to indicate pregnancy. The study reveals that the green bean seed germination test demonstrates higher accuracy, reaching up to 100%, with a sensitivity of 78.57%. This method is easier to conduct and more cost-effective when compared to rectal palpation. It provides an effective, efficient, and non-invasive alternative for pregnancy detection in livestock, proving advantageous for farmers. These findings have attracted significant attention within the agricultural community, highlighting more dependable and efficient techniques for determining buffalo pregnancy. This innovation has the potential to drive a substantial shift in livestock management practices, empowering farmers to improve animal welfare and overall production.

AUTHOR'S CONTRIBUTION

FLS, J, and NJ: Conceptualization. I and FLS: Data investigation. E: Data analysis. FLS: Writing the original article.

Conflict of interest

The authors have declared no conflict of interest.

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