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Evaluation of An ancient technique to diagnose the pregnancy in cattle using urine

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Diagnosing the pregnancy at an early stage is an important requirement for successful dairying and to improve the production of farmers. The available techniques of diagnosing pregnancy are laborious, costly & high technical such as rectal palpation and ultrasonography. On the basis of ancient Egyptian knowledge, a simple non invasive bioassay has been developed to diagnose the pregnancy called PUNYAKOTI Test (Veena et al, 1997). The objective of the present study was to develop farmer friendly simple and non invasive pregnancy diagnosis test for cattle. Urine samples of pregnant and non pregnant cattle's were collected randomly. Germination tests for Mung beans were performed by diluting the urine with distilled water ratio of 1:4, 1:10 and1:14(Veena et.al, 2003). Measurements of germination after 24 and 48 hours and shoot lengths were recorded with in 05 days, respectively. Distilled water was served as a control. General linear model analysis showed that urine of pregnant animals has inhibitory effect on seed germination and Shoot length which were significant (P=0.05) than non pregnant cows. It has been identified the concentration of a plant hormone known as Abscisic acid is higher in the urine of pregnant cows (170.62 nanomoles/ml of urine) than non pregnant cows(74.46 nanomoles/ml) (Veena et al, 2003).Main effect of Abscisic acid is to maintain the dormancy of the seeds. In conclusion, using above functional property of Abscisic acid can be developed farmer friendly simple and non invasive pregnancy diagnosis test for cattle using urine.

Key words: Cattle urine, Pregnancy diagnosis, Punyakoti test, Seed germination, Shoot length

Sri Lanka being a land of agriculture, dairy sector plays an important role among the all livestock sub sectors. Milk is an important food item. It plays key role in infant feeding, alleviating nutritional poverty and also dairy industry is important and has tremendous potential in developing the economy in the country, and it provides extensive employment opportunities. Therefore Government gives high priority to reaching self sufficiency in milk production.

The domestic milk production only constitutes about 17 percent of the requirement and the rest is imported. The government attention is most focused on the dairy sub sector; to develop this sector into a *'local viable industry'.* Dairy production plays a vital role in maintaining sustainability and crop yields in most smallholder mixed farming systems and has provided them with a source of regular daily income and a way of cushioning the risk of frequent crop and marketing failures. It also converts resources such as surplus green forage and crop residues available in and around household into cash products. These resources have limited alternative uses and the opportunity cost can be considered near zero (SAEC 1998).

Recent negative growth rates of cattle population may become a serious constraint for future dairy development in the country (DAPH 1999). Cattle breeding have been recognized as a critical issue for the dairy sector (MLDRI 1995). Because of difficulties in heat detection and pregnancy diagnosis of cattle. At present there are no simple laboratory tests available to diagnose pregnancy in cattle and buffaloes. The only method widely used at the farm level is the rectal palpation. But, this method has it own disadvantages. Therefore it is important to develop a simple test to detect the pregnancy in cattle.

Pregnancy diagnosis is an important requirement to increase the productivity of cattle and the income of the farmers. Economic losses are reduced by early identification of non pregnant farm animals, which can either be rebred or culled. In cattle, some of the currently available pregnancy tests such as ultrasonography, **Use of Milk or Plasma Progesterone are** highly technical**.** The main disadvantages of the use of ultrasonography are related to cost and time involved with the use of this technique. Highly sensitive quantitative assays, such as Radio immunoassay, must be carried out in licensed laboratories. **Estrous detection and rectal palpation are the widely used methods at the farm level.** The main disadvantage of rectal palpation is that it cannot be performed until later gestation. Usually rectal examinations take place between 45 and 60 days after insemination.

On the basis of ancient Egyptian technique that was practiced in Egyptian civilization during 2200 BC, farmer friendly simple technique has been develop to diagnose pregnancy called PUNYAKOTI Test (Veena et al, 2003). At that time this was practiced to diagnose the pregnancy in women by treating wheat and barley seeds with urine of suspected women for pregnancy. The woman suspected of being pregnant was asked to urinate on cotton bags containing wheat and barley seeds. The woman was diagnosed as pregnant if the seeds germinated and as non pregnant if they did not. At present this test was extended to make it suitable for diagnosis of pregnancy in farm animals.

In the urine of cattle, it has been identified a plant hormone known as Abscisic acid (ABA) apart from the normal urinary constitutes such as urea and uric acid. Abscisic acid is a single compound unlike the auxins, gibberellins, and cytokinins. It was called "abscisin II" originally because it was thought to play a major role in abscission of fruits. At about the same time another group was calling it "dormin" because they thought it had a major role in bud dormancy. A high concentration of ABA is found in the urine of pregnant cows (170.62 nanomoles/ml of urine) as compaired with that in the urine of non pregnant cows (74.46 nanomoles/ml) (Veena et al, 2003). It ABA result in decreased germination and shoot growth of seeds like paddy, mung beans etc. This functional property used in Punyakoti test, at the same time this can be easily carried out by dairy owners or farmers in rural areas by them selves.

METHODOLOGY

Study Design and Study Period

This study was conducted as an experimental study, in laboratory of Livestock and avian Sciences, during the period of August to December,

2008 to develop farmer friendly simple and non invasive pregnancy diagnosis test for cattle.

Study Population

Apparently healthy cattles were in pregnancy stage were selected purposefully, from peradeniya university farm respectively.

The pregnant cows were grouped into three groups by considering the pregnancy length as (a) where the pregnancy length is less than 03 months (b) pregnancy length is in between 03 and 06 months and (c) pregnancy length is more than 06 months. Sample preparation of urine was performed by diluting the urine with distilled water ratio of 1:4, 1:10 & 1:14 (Veena et al, 2003). 15 ml of diluted urine (1 ml urine made in to 15 ml with distilled water) was added to each Petri dish containing 25 mung seeds after half an hour distilled water soaking. Two Petri dishes were maintained for each cow. A control group was maintained using distilled water.

Data Collection

Germination percentages and Shoot length of Mung beans were measured and recorded

Germination percentage

Number of seeds germinated was count after 24 and 48 hours. Measurements were recorded and calculate the percentage.

Shoot length

Shoot length was measured using thread at 24 hour interval up to five days. .

pH determination

The pH of urine samples was by using digital pH meter.

Data Analysis

Statistical analysis

General linear model analysis was performed for all data to determine whether there is effect of urine of pregnant and non pregnant cattles. One way ANOVA test was used to compare the means of different treatments. The level of significance was set at <0.05 for all analyses.

RESULTS

Table 01: Germination (%) of Mung beans with different dilutions of urine of pregnant and non-pregnant cows

Treatments (cattle urine)	Germination (%) 1:4 Mean	1:4 SD	1:10 Mean	1:10 SD	1:14 Mean	1:14 SD
Non pregnant	32	2.94	42.7	49.44	64.75	6.89
pregnant						
< 03 months	0	0	42.5	49.08	48.25	8.65
03-06 months	7	1.15	44.5	51.39	56.50	6.35
> 06 months	0	0	42.25	48.80	54.25	6.84

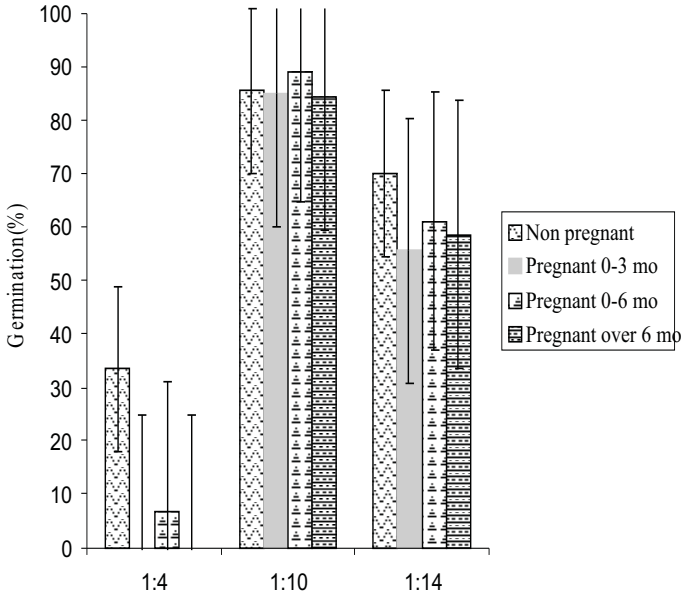

Figure 01: Germination (%) of Mung beans with different dilutions of urine of pregnant and non-pregnant cows

Table 02: Shoot length of Mung beans with different dilutions of urine of pregnant and non-pregnant cows

Treatment ratio	Shoot length (cm) Mean	SD
1:4	0	0.00
1:10	2.52	0.91
1:14	2.32	1.52

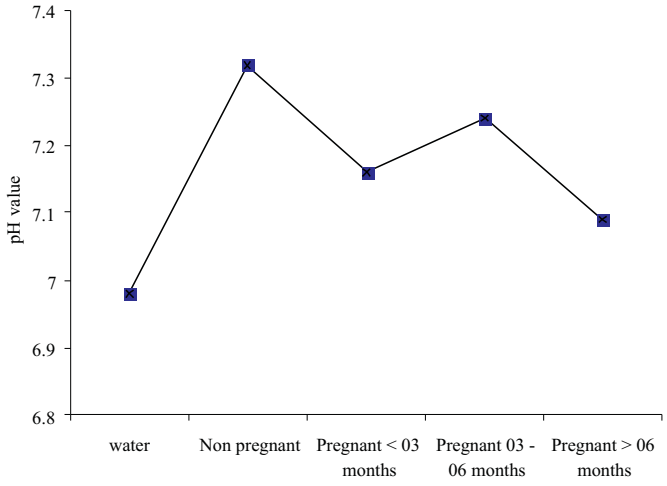

Figure 02: pH values of water and cattle urine samples.

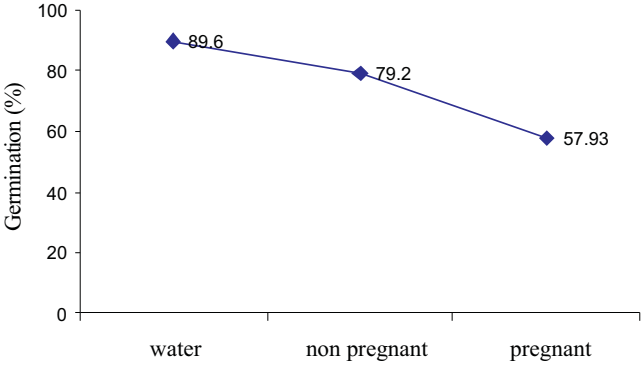

Figure 03: Germination (%) of Mung beans treated with water, urine of pregnant and of non-pregnant cows.

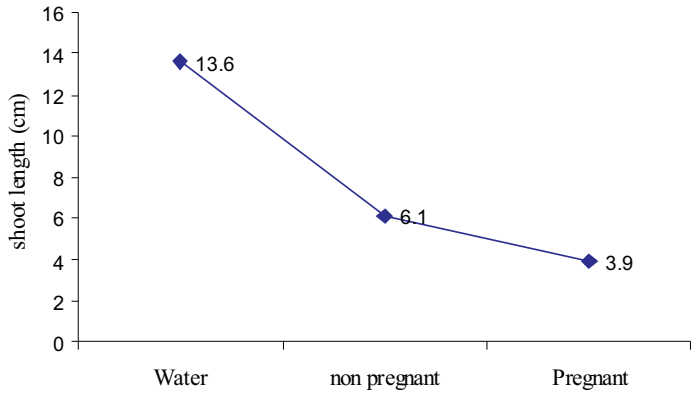

Figure 04: shoot length (cm) of Mung beans treated with water, urine of pregnant and of non-pregnant cows.

Table 03: Germination (%) of Mung beans after 24 hours, treated with water, urine of pregnant and non-pregnant cows

Treatments	Germination (%)	
	Mean	SD
Water	79.5[a]	17.8
Urine		
Non pregnant cows	68.2[b]	18.4
Pregnant cows		
< 03 months	40.0[c]	18.8
03-06 months	43.4[c]	18.7
> 06 months	40.7[c]	18.9

Means with different subscript are significantly differ.

Table 04: Germination (%) of Mung beans after 48 hours, treated with water, urine of pregnant and non-pregnant cows

Treatments	Germination (%)	
	Mean	SD
Water	89.6[a]	17.8
Urine		
Non pregnant cows	79.2[b]	18.4
Pregnant cows		
< 03 months	52.6[c]	18.8
03-06 months	60.6[c]	18.7
> 06 months	60.6[c]	18.9

Means with different subscript are significantly differ.

Table 05: Shoot growth of Mung beans treated with the urine of pregnant and non-pregnant cows

Treatments	Shoot length (cm)	
	Mean	SD
Water	7.6[a]	3.5
Urine		
Non pregnant cows	3.6[b]	3.2
Pregnant cows		
< 03 months	2.0[c]	3.2
03-06 months	2.2[c]	3.2
> 06 months	2.2[c]	3.2

Means with different subscript are significantly differ.

DISCUSSION

The Sri Lankan dairy industry is important and has tremendous potential in developing the economy in the country. Diagnosing the pregnancy of cows at an early stage is of considerable economic value in dairy management. The currently available laboratory techniques of diagnosing pregnancy in cattle are laborious, costly and non practicable. This study was an experimental study designed to develop the farmer friendly pregnancy test for cattle.

To develop this simple test, as materials only need seed sample like Mung beans and diluted cattle urine. The average germination percentage of Mung bean sample was 84%. The germination percentages of Mung beans treated with cow urine reduced significantly compared to those treated with water (Table 04). Among the dilution series of 1:4, 1:10 and 1:14, 1:14 was showed the significant difference in germination among the treatment groups' results. According to the figure 01 there was a significant difference (P=0.05) between germination percentages of pregnant and non pregnant in 1:14 dilution group. Significance difference in shoot length also observed in 1:14 dilution ratio (Table 02).As a result 1:14 was the dilution ratio used in experiment. The urine of the all categories was found to have similar pH values indicating that the suppression of seed germination and shoot growth is not due to pH (figure 02). However the urine of the pregnant cows suppressed the seed germination (57.93%) significantly than the non pregnant (79.2%) (Figure 03). The recorded Shoot length was significantly less when treated with the urine samples collected from pregnant cows (mean shoot length 3.89, SD3.16) than when compared to that of non pregnant cows (mean 6.1, SD 3.24) and water which was used as control treatment (mean 13.6, SD 3.41) (figure 04) Germination percentages and shoot growth were not significantly differing with in the pregnant group (Table 04 and 05). Germination percentages have same effect after 24 and 48 hours with water and cattle urine. (Table 03 and 04).

This simple test can be performed in field level at the same time by rural dairy farmers.

CONCLUSION

The germination percentages and shoot length of Mung beans treated with cow urine reduced significantly compared to those treated with water. Urine of pregnant cows dramatically inhibited the germination and shoot growth of mung beans than the non pregnant cows. This inhibitory effect persists throughout the pregnancy. In cattle urine apart from the normal urinary constituents such as urea and uric acid, a plant hormone known as Abscisic acid (ABA) has been identified. Its main effect on seeds is to maintain their dormancy. A high concentration of ABA acid is found in urine of pregnant cattle. (Veena et al, 2003). This simple test based on this hormone. This modified seed germination test is simple, non invasive, from the animal point of view, and does not require any chemicals or sophisticated instruments.

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