

### Maize seed germination inhibition test for pregnancy diagnosis in Zebu cows

\*<sup>1</sup>Okunlola, O. O., <sup>2</sup>Olorunnisomo, O. A., <sup>1</sup>Alalade, J. A., <sup>1</sup>Oyinlola, O. O., <sup>1</sup>Amusa, H. O.,  
<sup>1</sup>Adebisi, I. A. and Emiola, C. B.

<sup>1</sup>Department of Animal Health and Production Technology, Oyo State College of  
Agriculture and Technology, Igboora



<sup>2</sup>Department of Animal Science, University of Ibadan, Nigeria

\*Corresponding author: Olujuwonokunlola@yahoo.com; +2348064281822

#### Abstract

*Pregnancy diagnosis is an important part of good management and helps to maximize reproductive performance. Over time, different methods for detection of pregnancy in cows and other female domestic animals have been developed which include observation, physical examination, chemical tests and electronic instruments. Seed germination inhibition technique was carried out to detect pregnancy in Zebu cows. The urine samples collected from four pregnant cows, four non pregnant cows, four bulls and water (control) were subjected to seed germination test using maize seeds. Urine was collected between 5 and 7 am. Pregnant cows were confirmed by rectal palpation. The pH of the urine samples was determined before and after dilution and consequently, the urea concentration was also analyzed in the laboratory. The urine was diluted at the ratio of 1:4 with distilled water. Control test was also carried out with the addition of water only to the maize seeds. In each sterile Petri dish fifteen maize seeds were taken on the filter paper and 15mL of diluted urine was added. The seed germination inhibition percentages were observed after five days while shoot length growth inhibition was also observed after five days. Germination inhibition percentage of pregnant cows (64.16%) was significantly ( $P < 0.05$ ) higher compared to non-pregnant cows (34.18%), bull urine and water control. Shoot length of seeds germinated with distilled water had the highest value (4.38cm), seeds germinated with bull urine and non pregnant cow urine showed similarities while seeds germinated with pregnant cow urine had the least value (1.37cm) across the observations for shoot length growth inhibition. The result on pH showed clear differences in the pH of urine from pregnant (8.11- 8.25), non pregnant cows (6.05-7.04) and bull urine (6.35-7.34). The pH of the urine samples for non-pregnant cow and bull urine samples were similar to that of the control. Urea concentration of pregnant cow urine (11.06 mg/dL) was higher than that observed in bull urine 4.56 mg/dL and non pregnant cow urine 1.39 mg/dL. The study was able to validate that at 60% and above inhibition there is an indication of pregnancy while inhibition less than 60% shows the cow is non pregnant. Increased mean germination inhibition percentage and reduced shoot length in pregnant cow urine were indicative of pregnancy state.*

**Keywords:** Zebu cows, Pregnancy detection, Seed germination inhibition, Urine

#### Introduction

Early detection of pregnancy in cattle is of economic importance. Even though techniques such as rectal palpation, radiography, ultrasound technique, progesterone assay and rosette inhibition test are some of the pregnancy detection tests (Jainudeen and Hafez, 1993, Wani *et*

*al.* 2003), but these techniques needs laboratories facilities, professional experience, are invasive and expensive. Simple, non- invasive and non- expensive pregnancy test should be identified and utilized. Veena and Narendranath (1993) extended an ancient Egyptian pregnancy test of humans to cattle to diagnose

### *Maize seed germination inhibition test for pregnancy diagnosis in Zebu cows*

pregnancy by differential seed germination and based on the results they suggested that this test could be used as a simple test to diagnose pregnancy in cattle. The seed germination inhibition test is recognized as a door step technology to the farmers that can be done at farmers houses by the farmers since it requires inexpensive materials and does not require special skills. A simple seed germination technique comprises observation of differential seed germination response of wheat seeds to diluted fresh urine samples as reflected by significant inhibition of germination percentage in pregnant cow urine when compared to non pregnant cow urine (Veena and Narendranath, 1993). A papyrus in one of the earliest writing record of a urine-based pregnancy test in an Egyptian document described a test in which a woman who might the pregnant could urinate on wheat and barley seed over the course of several days, 'if the barley grows, it means a male child. Testing of these theory in 1963 found that 70 percent of the time the urine of pregnant women did promote growth, while the urine of non pregnant women and men did not (Allison, 2007). However, when similar test was conducted with urine of pregnant cows, it was observed to inhibit rather than promoting the growth of seeds. Veena *et al.* (2003) identified that the concentration of the plant hormone known as abscisic acid is higher in the urine of pregnant cows (170.62 nanomoles/ml of urine) than in non-pregnant cows (74.46 nanomoles/ml). this could be responsible for the inhibition of seeds treated with pregnant cow urine. The auxins, abscisic

acid, estrogen and progesterone in the pregnant cow's urine seem to be responsible for inhibition of germination and shoot length growth in wheat seeds (Veena and Narendranath 1993; Shinde, 1998 and Avahikar *et al.*, 2002).

#### **Materials and methods**

##### ***Study location and duration***

The study was carried out at the Dairy Unit, Teaching and Research Farm, University of Ibadan, Oyo State, Nigeria.

##### ***Animal selection and their general management***

Four Zebu bulls and Eight Zebu cows (four pregnant and four non pregnant cows) were selected from the University of Ibadan, Dairy Farm. The age of the animals ranged from 3 to 6 years. The animals were semi intensively managed, fed silage (a combination on elephant grass, spent grain and cassava peel) and were also allowed to graze.

##### ***Seed selection***

**Tropical maize (suwan- ISR yellow maize variety) were used for the study.**

##### ***Preparation of instrument***

The petri dishes, beaker, pipette were properly cleaned and sterilized in the oven. The

Maize seeds were taken and uniformly spread over a single layer filter paper bed in sterile Petri dishes.

##### ***Experimental design***

Maize Seed was used for this experiment (water-control, bull urine, urine for pregnant and non-pregnant cattle). Below is the lay out of the experiment.

#### **Layout of the experiment**

<b>BREED</b>	<b>NOS</b>	<b>NOA</b>	<b>REP</b>	<b>NOB</b>	<b>OD</b>
Control	15	0	10	40	5
BU	15	4	10	40	5
PCU	15	4	10	40	5
NPCU	15	4	10	40	5

Breed = Zebu, Control = Distilled water, NOS = number of seed, NOA= number of animals, NOB= observations, OD = observation da ys, BU = bull urine, PCU = pregnant cow urine, NPCU = non pregnant cow urine.

### **Urine selection**

The urine samples used for detection of pregnancy exhibited by the inhibition of seed germination were collected from inseminated and non-inseminated cows. The samples were collected during natural micturition, milking and other time by continuous stroking of the skin just below the vulva of cows to induce urination. Pregnant cows were confirmed by per rectal palpation. The urine was collected early in the morning between 5.00 to 8.00 am. Bull urine serve as BU, Inseminated cows served as PCU and the urine from non inseminated cows served as NPCU. A clean, dry plastic container was used to collect the urine and was transferred into a bottle immediately. The collected urine was subjected to seed germination test within four to six hours of collection.

### **Seed germination inhibition test**

A simple seed germination technique comprised observation of differential germination response and shoot length growth of maize seeds in diluted fresh urine samples as reflected by significant inhibition of germination percentage in bull urine, non-pregnant cow urine when compared to pregnant cow urine.

### **Test procedure**

In the laboratory, the urine was diluted at the ratio of 1:4 with distilled water. The germination inhibition test observed by adding four types of sample. They were;

**Sample 1-** test was carried out with the addition of water only to the Maize seeds

**Sample 2-** urine from bulls and distilled water

**Sample 3-** urine from non pregnant cows and distilled water

**Sample 4-** urine from the cows, confirmed pregnant and distilled water.

In each sterile Petri dish, 15 maize seeds were taken on the filter paper and 15 mL of diluted urine was added. The Petri dishes were covered with the trays to avoid

evaporation, wherein there was little air movement at the bottom of the inverted trays and they were undisturbed for five days. The percentage of seed germination in seeds individually was taken on the fifth day. On the seventh day, the shoot length in centimeters (cm) of germination seed was taken according to the procedures of Dilrukshi *et al.* (2009).

### **Germination inhibition percentage**

Data on germination inhibition was recorded on the 5<sup>th</sup> day. The number of ungerminated seeds was counted and percentages was calculated using the following formula;

Germination inhibition percentage was calculated by

$$\frac{\text{Number of seeds not germinated}}{\text{Number of seeds put in petri dishes}} \times 100$$

### **Statistical analysis**

The data was analyzed to observe any significant differences in the above parameters among four groups. All data (seed germination inhibition percentages and shoot length growth) were expressed as Mean  $\pm$  SE using one-way ANOVA. Treatment means will be compared using Duncan Multiple Range Test, as outlined by SAS (1995)

### **Results and discussion**

The mean germination inhibition percentage of maize seeds in different groups at the same day of urine collection. Germination inhibition percentage of distilled water (DW), bull urine (BU), non pregnant cow urine (NPCU) and pregnant cow urine (PCU) were 13.33 $\pm$  1.52, 28.75 $\pm$ 1.63, 34.18 $\pm$ 1.81 and 64.16 $\pm$ 2.58 respectively. It was observed that the germination inhibition percentage of urine of pregnant cows is significantly ( $P < 0.05$ ) higher from the Non pregnant cows, Bull and Water control groups. But there was no statistical significance between Non pregnant cows, Bull and Water control

### *Maize seed germination inhibition test for pregnancy diagnosis in Zebu cows*

groups. The mean values of shoot length (cm) inhibition of maize seeds on the 5<sup>th</sup> day of observation. Shoot length (cm) of distilled water (DW), bull urine (BU), non pregnant cow urine (NPCU) and pregnant cow urine (PCU) at day five were  $4.38 \pm 0.04$ ,  $3.00 \pm 0.05$ ,  $2.73 \pm 0.07$  and  $1.37 \pm 0.16$  respectively. Significant difference ( $P < 0.05$ ) was observed among water, bull, non-pregnant cows and pregnant cows. Seeds germinated with distilled water had the highest value, seeds germinated with bull urine and non pregnant cow urine showed similarities while seeds germinated with pregnant cow urine had the least value across the observations. The hydrogen ion concentration was evaluated for the germination fluids as presented in Figure 1. Results showed that pH values of urine differ from each other even when compared to the control. Urine from pregnant cow had the highest values of 8.11 and 8.28, non pregnant 6.05 and 7.04, bull urine 6.35 and 7.34 before and after dilution, respectively. The pH value for control (water) remained constant at 7.00.

The urea concentration of the urine samples was evaluated as presented in Figure 2. The urea concentration of pregnant cow urine (11.06 mg/dL), non pregnant cow urine (1.39mg/dL) while bull urine was (4.56mg/dL). The seed germination inhibition test is recognized as a door step technology to the farmers that can be done at farmers houses by the farmer since it requires inexpensive materials and does not require special skills. Early pregnancy diagnoses in cows of economic importance in dairy cattle management in Nigeria. The techniques of diagnosing pregnancy in cows are expensive, invasive and not practicable by local cattle farmers. The techniques require professionals like veterinarians which is laborious and expensive. The current study provided a

simple, economical, noninvasive and inexpensive method of pregnancy detection in Zebu cows. The urine of pregnant cows significantly inhibited the germination of maize seeds on the 5<sup>th</sup> day compared with Non pregnant cows, bull and Water control groups. The urine of pregnant cows also significantly inhibited the shoot length of maize seeds compared with Non pregnant cows, bull and Water control groups. The seed treated with diluted urine of test pregnant animals, a gradual discoloration of seeds as well as the germination fluid set in after 48 hours, which deepened with the passage of time. This indicated that the urine of pregnant cows contains some metabolites and substances which inhibit the germination and shoot length of maize seeds. Hormone metabolites excreted through urine might affect the seed germination in pregnant cow urine. With reference to the factor that might be influencing such a differential response in urine treated seeds, Veena and Narendranath (1993) opined that plant growth regulators such as auxins are also excreted in high concentrations in urine during pregnancy in cows which might be causing inhibitory responses to seed germination and shoot growth. Germination inhibition percentages of pregnant cows were significantly higher compared to non-pregnant cows, bull and Water control groups at day 5 as shown in Table 1. On the other hand, shoot length growth inhibition of pregnant cows, were significantly higher compare to Non pregnant cows, bull and Water control groups as shown in Table 2. Increased mean germination inhibition percentage and reduced shoot length in Zebu cows was indicative of pregnancy state. The present finding is in agreement with the observations of Veena and Narendranath (1993); Veena *et al.* (1997); Shinde (1998); Avahikar *et al.* (2002), in cows and

buffaloes, Yadav (1999) in does and ewes. The trend observed with the shoot length on Table 2, could be attributed to plant growth which is influenced by a number of factors. The growth of the plants can also be influenced by certain chemicals resembling plant hormones both in structure and functions. These are called growth regulators. Growth regulators are chemical substances, other than naturally produced hormones, which promote, inhibit or modify growth and development in plants. The naturally produced growth hormones are broadly grouped under five major classes. They are (i) Auxin(ii) Gibberellins (iii) Cytokines (iv) Ethylene (v) Abscissic acid (Schopfer *et al.*, 1979). For the pregnancy diagnosis in cows by seed germination test, abscissic acid, auxin, estrogen, and progesterone have great roles in the urine of pregnant cows (Veena and Narendranath 1993; Shinde, 1998; Avahikar *et al.*, 2002). The action mechanism of abscissic Acid (ABA), a potent physiological inhibitor of seed germination, is not yet understood. Schopfer *et al.* (1979), suggested that the hormone induces seed dormancy by restricting water uptake of the imbibed seed during a critical period of germination, where an active push of embryo expansion was needed to support embryo growth. Higher concentration of abscissic acids leads to low germination of seed and shoot length. Estrogen and progesterone in their natural forms did not affect either the seed germination or shoot length. But according to the study conducted by Veena and

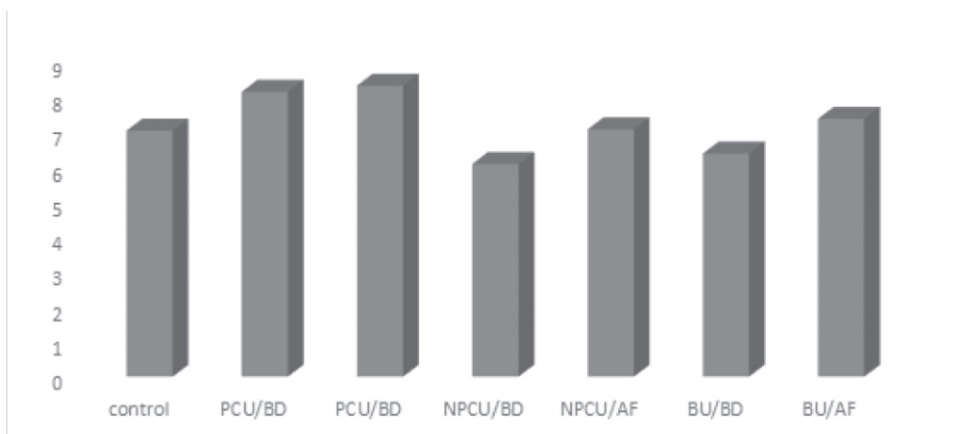
Narendranath (1993), both wheat and green gram seed germination rate was significantly ( $P < 0.01$ ) decreased in diluted pregnant cow urine compared to non pregnant cow urine. The result on pH showed clear differences in the pH of urine from pregnant, non pregnant cows and bull urine with higher values in the pregnant than non-pregnant and bull urine group. The pH of the urine samples for non-pregnant cow and bull urine samples were similar to that of the control. This observation is partially in agreement with the findings of Diluskshi *et al.* (2009). These recent findings only support the report of pH for non-pregnant urine samples but not in agreement with that observed in pregnant cattle urine. The pH range of the urine samples used for the seed germination inhibition test are in a range of neutrality to slightly alkaline in nature, hence, the inhibition of seeds is not attributed to the pH of the urine samples because the specified range is tolerable for the seeds germination. This is in conformity with the assumption of Dilrukshi *et al.* (2009); Veena and Narendranath (1993) respectively. Results on urea concentration of pregnant cow urine (11.06 mg/dL) was higher than that observed in bull urine and non pregnant cow urine (4.56 mg/dL and 1.39 mg/dL), respectively. Nagaraja (2012) in his short communication reported that inhibitory effect of the seeds may be attributed to the relatively higher concentration of urea and abscissic acid found in the urine samples of pregnant cows. These current findings on urea concentration is also in agreement with his communication.

**Table 1: Mean values of germination inhibition percentage and Mean shoot lengths (cm) of maize seeds in different groups at day 5**

Parameter	Water n=40	BU n=40	NPCU n=40	PCU n=40
Germination Inhibition Percentage	13.33±0.04 <sup>b</sup>	28.75±1.63 <sup>b</sup>	34.18±1.81 <sup>b</sup>	64.16±2.58 <sup>a</sup>
Mean shoot length	4.38±1.52 <sup>a</sup>	2.73±0.07 <sup>b</sup>	3.00±0.05 <sup>b</sup>	1.37±0.16 <sup>c</sup>

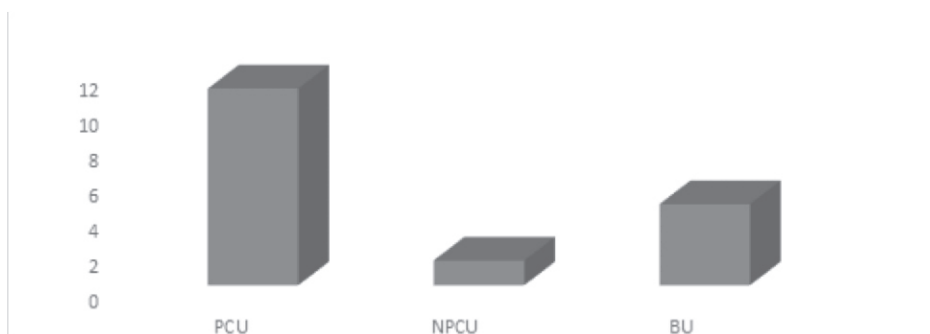
a,b,c = means on the same row with different superscripts are significantly different ( $P < 0.05$ ). control = Distilled water, BU = Bull urine, NPCU= Non pregnant cow urine, PCU= pregnant cow urine

*Maize seed germination inhibition test for pregnancy diagnosis in Zebu cows*



**Fig. 1: pH values of water and urine samples**

Control = Water, PCU/BU = pregnant cow urine before dilution, PCU/AF= pregnant cow urine after dilution, NPCU/BD = Non pregnant cow urine before dilution, NPCU/AF = Non pregnant cow urine after dilution, BU/BD= Bull urine before dilution and BU/AF = Bull urine after dilution



**Fig. 2: Urea concentration of urine samples**

PCU= Pregnant cow urine, NPCU= Non Pregnant cow urine, BU= Bull urine

**Conclusion**

The study showed that that maize seeds can be used for germination inhibition test for pregnancy diagnosis in Zebu cows. Urine of pregnant cows has an inhibitory effect on seed germination and shoot length than that observed with non pregnant cow, bull and water.

**References**

**Avahikar, G. T. 2002.** In 89th Session of Indian Sci. Congress Association held from 3rd -7th January, 2002 at

Lucknow.

**Dilrukshi, H. N. N. and Perera, A. N. F. 2009.** Evaluation of an ancient technique to diagnose pregnancy in cattle using urine. *Wayamba Journal of Animal Science*, 578, 06-08.

**Jainudeen, M. R. and Hafez, E. S. E. 1993.** Pregnancy Diagnosis. In: *Reproduction in Farm Animals*. 6th Edition, Lea & Fibiger, Philadelphia, USA, 446-459.

**Nagaraga, T. G. 2012.** A microbiologist

- view on improving nutrient utilization in ruminant animals. Proceedings 2012, Florida ruminant nutrition symposium, Gainesville, Florida, USA.
- Schopfer, P. D., Bajracharya, C. and Plachy, D. 1979.** Control of seed germination by abscisic acid. I. Time course of action in *Sinapis alba* L. *Plant Physiology* 64, 822-827.
- Shinde, A. D. 1998.** M.V.Sc., dissertation, Maharashtra Agricultural University, Parbhani, India.
- Allison, T. 2007.** A History of the Pregnancy Test or Is the Rabbit in Heat? Accessed: November 18, 2007.
- Veena, T. and Narendranath, R. 1993.** An ancient Egyptian pregnancy test extended to cattle. *Current Science Journal*, 65, 989-990.
- Veena, T. 1997.** Contraceptives and Delivery Systems, 13, 49-53.
- Wani, N. A., Mustafa, S., Misra, A. K. and Maurya, S. N. 2003.** Pregnancy diagnosis in farm animals – a review. *Indian Journal Dairy Sciences.*, 56 (1), 1-8.
- Yaday, C. L. 1999.** Variations in resistance to haemonchosis: selection of female sheep resistant to *Haemonchus contortus*. Proceedings of 86th Session of Indian Science Congress held at Chennai, 3rd -7th January, 1999.

*Received: 7<sup>th</sup> April, 2019*

*Accepted: 25<sup>th</sup> July, 2019*