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SHORT COMMUNICATION:

Early Pregnancy Diagnosis in Cows Using Germination Responses of Different Crop Seeds to Urine Treatment

Alganesh Tola, GebreEgziabher GebreYohannis, Mulugeta Kebede, Gizaw Kebede, Chernet Asfaw, Jiregna Dessalegn and Ulfina Galmessa

Bako Agricultural Research Center, P.O. Box 03, west Shoa, Ethiopia

Abstract

This study tested pregnancy in cows using differences in germination rates of five crops (maize, wheat, barely, field pea and sorghum) mixed with urine from 3 inseminated and 5 non-inseminated cows. Germination was tested using 25, 50, 75 and 100 % diluted urine There was no germination in 100 and 75% urine in both cases. At 50% dilution, urine from pregnant cows inhibited germination of all crops, but urine from non-pregnant cows inhibited germination of barely, wheat and sorghum and favoured germination of maize and field pea The result showed that germination was affected ($P < 0.001$) by treatment and crop type only. The study indicated that the test could be performed starting from one month of pregnancy using maize and field pea at 50% urine dilution and field pea, sorghum and wheat at 25% urine dilution. Further detailed studies are required, including other species of farm animal, to develop application methods of this simple technology at smallholder farm conditions.

Introduction

It is important to make the right pregnancy diagnosis as soon as possible after insemination so that non-pregnant animals can be observed more closely for heat (Heinonen, 1989). Pregnancy diagnosis in cattle is often done by rectal palpation requires skill and experience and is performed two months after breeding. The ideal pregnancy test would be one that is inexpensive, accurate and easily applicable that could be implemented under farm conditions as early as 17-19 days post breeding. There was an attempt by ancient Egyptians to diagnose pregnancy in cattle using a test that relies on the differential response in germination and shoot growth of wheat and barley seeds to the urine of pregnant and non-pregnant cows (Veena and Narendranath, 1993)..

The objective of this study was to test the validity of the technology in Horro cows using different crops

Materials and Methods

The study was conducted at Bako Agricultural Research Center which is located 258 km west of Addis Ababa at an altitude of 1650 masl. Urine from 3 inseminated and 5 non-inseminated Horro cows was used for the study. Urine was collected once a month for three consecutive months from cows inseminated and confirmed pregnant at the end of third month. The non-inseminated cows had at least three months after parturition. Five ml of urine solution from these two group of cows was prepared with tap water at 25%, 50%, 75% and 100 % urine, and distilled and tap water were used as control.. Clean seeds of five crops (maize, wheat, barley, sorghum and field pea) were used to test germination rates.

Ten treatments were formed by combining four dilutions (25, 50, 75 and 100% urine) and two urine sources (pregnant and non pregnant cows) and an additional two control treatments (distilled and tap water). Enough amount of the treatment solution was applied to petridishes containing twenty seeds of each crop. The treatments were replicated 6 times. The experiment was done three times at the first, second and third month of pregnancy stages. Germination of the seeds was checked for one week. After one week, the germinated seeds were counted and recorded. The treatment combinations are presented in Table 1.

Table 1. Treatment combinations

Treatment	Dilution (% Urine in solution)	Urine source
1	100	Pregnant cow
2	100	Non pregnant
3	75	Pregnant cow
4	75	Non pregnant
5	50	Pregnant cow
6	50	Non pregnant
7	25	Pregnant cow
8	25	Non pregnant
9	Tap water	Control
10	Distilled water	Control

The germinated seeds were counted from week one and percentage germination calculated and the data were transformed to their arc-sign equivalent. The General Linear Model of the Statistical Analysis System

(SAS, 1996) was used for data analysis. To determine the stage of pregnancy at which the method could be used to test pregnancy, data collected from pregnant cows were reduced to four (T1, T3, T5 and T7). The data were then transformed back to percentages .

Results and Discussion

Treatment, crop type, month, and treatment x crop type interaction significantly ($P<0.001$) affected germination percentage (Table 2). Treatment (T) with 100 and 75% urine (T1 to T4) resulted in no germination of the crops (Table 3). The highest germination was observed for distilled ($87.9 \pm 0.06\%$) and tap water ($88.5 \pm 0.06\%$). Germination increased as the proportion of urine from both pregnant and non-pregnant cows in the solution was reduced. In both 50% (T5 and T6) and 25% (T7 and T8) urine dilutions, urine from non-pregnant cows significantly reduced germination compared to urine from pregnant cows.

Comparison of the treatment x crop type interaction groups indicated that treatments 1 to 4 totally inhibited germination in all crops. At 50 % dilution, urine from pregnant cows inhibited germination of all crops, while urine from non-pregnant cows inhibited germination of barely, wheat and sorghum and favoured germination of maize and field pea. The germination of maize ($20.9 \pm 0.51\%$ vs $0 \pm 0.84\%$) and field pea ($12.2 \pm 0.51\%$ vs $0 \pm 0.84\%$) were higher ($P<0.001$) for urine from non-pregnant than pregnant cows. Thus, at 50 % dilution maize and field pea could be used as an indicator of pregnancy. At 25% dilution, urine from non pregnant cows resulted in a higher germination rate of field pea (50.9 vs 19.8%), wheat (78.2 vs 56.6%) and sorghum (19.6 vs 2.6%), respectively compared to urine from pregnant cows.

The effects of stage of pregnancy and its interaction with dilution rate were not significant ($P > 0.05$). The result indicated that the test could be performed starting from one month of pregnancy. A similar study in India by Veena and Narendranath (1993). showed that the urine of pregnant cows dramatically inhibited seed germination compared to that of non-pregnant cows and this persisted for 2-3 months after parturition. Generally, the inhibitory factor in cattle urine on seed germination has not yet been identified. The mammalian urine is known to contain auxins, the plant growth regulators, that have an equivocal effect on seed germination. Therefore, it is likely that the enhanced levels of such hormones in urine during pregnancy of cows might be causing the observed inhibition of seed

germination (Veena and Narendranath (1993). Thus, from this study it can be concluded that maize can serve as a good indicator of pregnancy using 50% diluted urine and field pea, sorghum and wheat at 25% urine dilution starting from first month of pregnancy.

Table 2. Least square mean (±SE) germination percentage of five crops using different treatments

Source	N	Germination	
		Transformed	Percent germination
		Mean ± SE	Mean ± SE
Treatments	8	***	***
100% urine from pregnant cow	3	0 ± 1.81 ^{e}	0 ± 0.1 ^{e}
100% urine from non pregnant cow	5	0 ± 2.34 ^{e}	0 ± 0.17 ^{e}
75% urine from pregnant cow	3	0.9 ± 1.81 ^{e}	0.2 ± 0.10 ^{e}
75% urine from non pregnant cow	5	0 ± 2.34 ^{e}	0 ± 0.17 ^{e}
50% urine from pregnant cow	3	10.9 ± 1.81 ^{d}	3.6 ± 0.10 ^{d}
50% urine from non pregnant cow	5	2.9 ± 2.34 ^{e}	0.26 ± 0.17 ^{e}
25% urine from pregnant cow	3	41.4 ± 1.82 ^{b}	43.7 ± 0.10 ^{b}
25% urine from non-pregnant cow	5	27.4 ± 2.34 ^{c}	21.1 ± 0.17 ^{c}
Tap water	6	69.6 ± 1.43 ^{a}	87.9 ± 0.06 ^{a}
Distilled water	6	70.2 ± 1.34 ^{a}	88.5 ± 0.06 ^{a}
Crop type		***	***
Barely	8	18.9 ± 1.40 ^{c}	105 ± 0.06 ^{c}
Field pea	8	20.3 ± 1.40 ^{bc}	12.0 ± 0.06 ^{bc}
Maize	8	23.3 ± 1.40 ^{b}	15.6 ± 0.06 ^{b}
Sorghum	8	18.7 ± 1.40 ^{c}	10.3 ± 0.06 ^{c}
Wheat	8	30.5 ± 1.40 ^{a}	25.8 ± 0.06 ^{a}
Month	8	***	***
1	8	21.85 ± 1.03 ^{b}	
2	8	19.60 ± 1.03 ^{b}	
3	8	25.53 ± 1.03 ^{a}	
Treatment X crop interaction		***	***

Means in a column within a group followed by different superscripts vary significantly (*** P<0.01)

Table 3. Analysis of variance for the effect of different stages of pregnancy on germination

Source	N	Arc Sign transformed	Percent germination
	3	Mean ± SE	Mean ± SE
Month	3	NS	NS
1	3	6.19 ± 1.86	1.2 ± 0.11
2	3	6.29 ± 1.86	1.2 ± 0.11
3	3	10.22 ± 1.86	3.2 ± 0.11
Month X dilution	3	NS	NS
1	3	0 ± 3.73	0 ± 0.42
3	3	0 ± 3.73	0 ± 0.42
5	3	0 ± 3.73	0 ± 0.42
7	3	4.00 ± 3.73	0 ± 0.42
11	3	4.77 ± 3.73	0.7 ± 0.42
12	3	36.13 ± 3.73	34.7 ± 0.42

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