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Pregnancy diagnosis in pigs using seed germination inhibition test: An evaluation study in Meghalaya

ASHOK KUMAR[1], R K DEWRY[2], RAM SINGH[3], P C JAT[4], MAHAK SINGH[5] and G KADIRVEL[6]

ICAR Research complex for NEH Region, Umiam, Meghalaya 793 103 India

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ABSTRACT

The present piece of study was carried out on 315 breedable sows of approximately 2–4 years of age irrespective of parity with good body condition in varying managemental conditions under subtropical rainfall areas of Meghalaya. These animals were fed on kitchen wastes with locally available green fodder. The animals those were non return after one month (range 28–34 days) post-breeding, were grouped as positive group (n=215), non-mated pigs as negative group (n=100) and distilled water as control group (n=100). Urine samples were collected in clean, sterilized plastic containers through manual method at farmer's door during natural micturition in the morning and diluted at the ratio of 1: 4 with distilled water for pregnancy diagnosis on the same day of collection. Wheat seed with average 85% of germination after 48 hrs at room temperature was selected for the entire study. In each sterile petri dish 15 wheat seeds were taken on the blotting paper and 15 ml of diluted urine was added and covered with trays to avoid evaporation and kept undisturbed for 48 hrs at room temperature. Test was conducted in triplicate for each animal. Control test was also carried out with the addition of distilled water only to the wheat seeds. The germination inhibition percentage and shoot length (cm) of germinated seeds in each petri dish was recorded and calculated at 48, 72 and 96 hrs after adding of the sample. All data were analysed using two-way ANOVA by SPSS software version 17 and expressed as Mean ± SE. The mean germination inhibition percentage of wheat seeds was significantly ($P<0.05$) differ between pregnant (75.66 ± 3.48), non pregnant (28.70 ± 2.96) and control (19.48 ± 2.69) groups. Shoot length (cm) was significantly less in pregnant when compared to non pregnant and control groups and also significantly differ within the group at different time intervals. Mean germination inhibition percentage and reduced shoot length in positive group was indicative of pregnancy status. Hence, it can be concluded that seed germination inhibition is a useful technique to detect pregnancy in pigs as a simple, non-invasive and cost effective method and must be popularize among the farmers, veterinary officers and paraveterinary workers for field level application.

Key words: Pregnancy diagnosis, Seed germination, Shoot length, Sow urine

North Eastern Himalayan (NEH) region shares 38.42% of country's total pig population (19th Livestock census-2012) and people of region are mostly (>90%) pork consumers, therefore, their livelihood and nutritional security is mainly depends upon piggery sector. When the time of rebreeding is reduced, the reproductive performance will be improved (Oltenacu *et al*. 1990). An accurate early pregnancy diagnosis is required to identify non- pregnant animals soon after breeding, is vital as it permits a timely rebreeding of non-pregnant animals with minimal loss of estrous cycles so that production time loss from infertility problems may be reduced by appropriate treatment or through culling of unproductive animals (Holness 1991, Lalrintluanga and Dutta 2009). In this respect, a plethora of pregnancy diagnosis techniques have been tried with variable degrees of success (Wani *et al*. 2003, Dilrukshi and Perera 2012), but an unique method of pregnancy diagnosis at the field level has not yet been established. Most of these techniques require extensive handling and restraining of the animal, expensive and sophisticated laboratory equipments, time consuming, highly technical and needs a specialist for interpretation, which are beyond the reach of resource poor farmers, especially in NEH region of India which make pregnancy diagnosis impractical at the farmer's door step ((Moriyoshi *et al*. 1996, Lalrintluanga and Dutta 2009). Rectal palpation is not practiced in pig, sheep/goat due to its smaller pelvic cavity. Ultrasonography is commonly used in the developed countries, are yet to gain wide acceptance in developing countries like India due to either too expensive to acquire and maintain or complicated for farmer, often poor illiterate pig producers (Ndu *et al.* 2000).

The ideal pregnancy test would be one that is economically affordable at farmer's forum, accurate and

Present address: [1] Scientist, Division of Livestock Production, [2]Research Associate, Division of Livestock Production, ICAR RC for NEH Region, Umiam. [3]Associate Professor, School of Social Sciences, CPGS, CAU, Barapani.[4]Senior Scientist, ICAR-ATARI, Zone-III, [5]Scientist, ICAR RC, Nagaland centre, [6]Principal Scientist and Head, Division of Livestock Production, ICAR RC for NEH Region, Umiam

easily applicable as early as 24–28 days post breeding. Therefore, there is a consistent effort in search of an alternative, simple, non-invasive, farmer's friendly and inexpensive technique for pregnancy detection in pigs. On the basis of ancient Egyptian technique that was practiced in Egyptian civilization during 2200 BC, farmer's friendly simple technique has been develop to diagnose pregnancy called PUNYAKOTI Test (Veena and Narendranath 1993, Dilrukshi and Perera 2012) in cattle using wheat and barley seeds. Seed germination inhibition test is simple, inexpensive, non- invasive, non-skilful test which can be used to diagnose pregnancy at any place inside the jungle or villages in the NEH regions (Perumal 2014) and has been recognized as a door step technology to the farmers. Since it require inexpensive materials and does not require special skills, hence, can be done at farmer's houses by the farmer itself. This test has been practiced in cattle, mare with varying degree of success, but there is a paucity of literature in swine and is a need of hours in the region. Hence, this piece of study was carried out during field visits to evaluate seed germination inhibition test for early detection of pregnancy in pig in Meghalaya at farmer's door step.

MATERIALS AND METHODS

Experimental animals: The study was carried out on 315 breedable sows (185 crossbred and 130 local pigs, Niang Megha) of approximately 2–4 years of age irrespective of parity with good body condition from the different villages of East Khasi Hills, Jayantia Hills and Ri-Bhoi districts of Meghalaya in varying managemental conditions during February, 2015 to January, 2016. These animals were fed on kitchen wastes with locally available green fodder. The animals those were non return after one month (range 28–34 days) post-breeding, were grouped as positive group (n=215), non-mated pigs as negative group (n=100) and distilled water as control group (n=100).

Collection of urine sample: The urine samples were collected in clean, sterilized and dry plastic containers through manual method at farmer's house during natural micturition in the morning and were used for pregnancy diagnosis on the same day of collection. Petri dishes, beaker, pipette etc. were properly cleaned and sterilized by heating at 121ÚC for 20 minutes. The laboratory work was carried out in the Division of Livestock Production, ICAR Research Complex for NEH Region, Umiam, Meghalaya. The pH of urine samples was measured by using digital pH meter.

Seed germination inhibition test: Urine samples were subjected to seed germination inhibition test within 4 to 6 hrs of sample collection. In the laboratory, urine was diluted at the ratio of 1: 4 with distilled water. Wheat seed samples procured locally which recorded an average 85% of germination after 48 h at room temperature was selected for the entire study. In each sterile petri dish 15 wheat seeds were taken on the blotting paper and 15 ml of diluted urine was added. For each sow, test was conducted in triplicate. Control test was also carried out with the addition of distilled water only to the wheat seeds in petri dish on blotting paper. Petri dishes were covered with trays to avoid evaporation wherein there was little air movement at the bottom of the inverted trays and kept undisturbed for 48 h at room temperature. After 48 h, the seeds were examined for germination inhibition percentage and shoot length in positive, negative and control groups (Veena and Narendranath 1993) and any kind of discolouration of either seeds or germination fluid or both after 48 hrs were recorded in each case. The germination inhibition of seeds in each petri dish was observed at 48, 72 and 96 h after adding of the sample. The shoot length of each germinated wheat seed was measured and recorded using thread in centimetres (cm) at 48, 72 and 96 h. Number of seed germination in each petri dish was recorded and calculated percentage as following formula:

$$\text{Germination inhibition percentage} = \frac{\text{No. of seeds not germinated in Petri dishes}}{\text{Total No. of seeds taken in Petri dishes}} \times 100$$

× 100

Statistical analysis: All data were expressed as Mean ± SE. The difference among the seed germination inhibition percentage and shoot length of different groups were analysed using two-way ANOVA by SPSS software version 17 and significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

The mean germination inhibition percentage (MGIP) of wheat seeds was significantly ($P<0.05$) differ between pregnant (75.66 ± 3.48), non pregnant (28.70 ± 2.96) and control (19.48 ± 2.69) groups (Fig. 1). The recorded Shoot length (cm) was significantly ($P<0.05$) less in pregnant when compared to that of non pregnant and control groups. Shoot growths were also significantly ($P<0.05$) differ within the group at different time intervals (Table 1). The water control test was performed as a comparative test to differentiate and visualize clear results between the test groups. The observations of this study were in conformity with Veena and Narendranath (1993), Veena *et al*. (1997), Avahikar *et al*. (2002) and Krishna and Veena (2009) where the authors found the similar results in cow, buffalo, goat and sheep.

Similarly, Swamy *et al*. (2010) and Islam (2013) observed that MGIP and mean shoot length of the

Table 1. Shoot length (cm) of germinated wheat seeds in pregnant (n=215), non-pregnant (n=100) and control (n=100) groups (mean ± SE)

Groups	Time (h)		
	48	72	96
Pregnant	0.41 ±.021^{Aa}	0.67±.036 ^{Ab}	1.09±.023 ^{Ac}
Non-Pregnant	1.36±.039 ^{Ba}	1.83±.043 ^{Bb}	2.75±.048 ^{Bc}
Control	3.37±.074 ^{Ca}	4.26±.097 ^{Cb}	6.52±.086 ^{Cc}

Means with different superscripts within columns (A, B, C) differ significantly ($P<0.05$)

Means with different superscripts within rows (a,b,c) differ significantly ($P<0.05$).

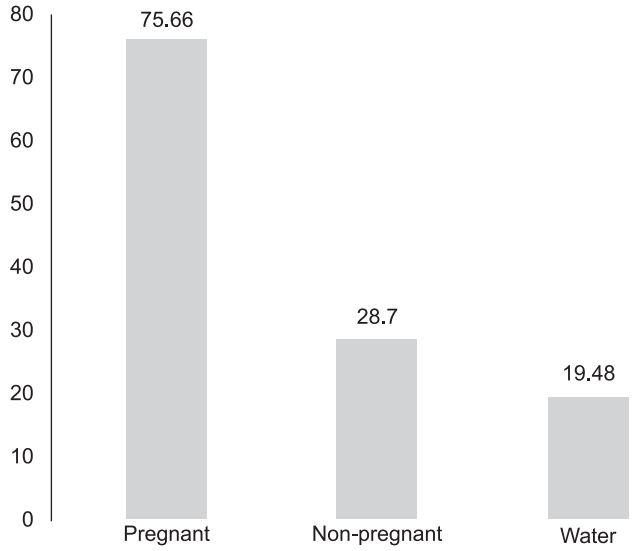

Fig. 1. Germination inhibition percentage of wheat seeds in pregnant, non-pregnant and control groups.

germinated wheat seeds significantly ((P<0.05)) differ in positive and negative group and concluded that seed germination inhibition technique is useful to detect pregnancy in Malnad Gidda cattle and sheep, respectively. Dilrukshi and Perera (2012) observed that the urine of the pregnant cows suppressed the mung beans seed germination and shoot length significantly than that of non pregnant cow and control group, are similar to present findings. Recently, Rine *et al*. (2014) also recorded that increased mean germination inhibition percentage and reduced shoot length of wheat seed kept in urine of post bred cow were indicative of pregnancy.

The urine of the all categories was found to have similar pH values indicating that the suppression of seed germination and shoot growth was not due to pH. Nirmala *et al*. (2008) concluded that estrogen and progesterone hormones did not influence seed germination and shoot length. The most probable factor influencing seed germination and shoot growth may be plant growth regulators such as auxins and abscicic acid which are excreted in high concentrations in urine of pregnant cows (Veena and Narendranath 1993). Inhibitory effect of pregnant cow urine on seeds starts by the end of the first month of pregnancy and persists till three months post partum, may be due to metabolites of progesterone, estradiol or some pregnancy associated substance as one of probable factors excreted in urine and exhibiting such an effect cannot be rules out. But whether and to what extent such a metabolite influences seed germination and shoot growth needs to be clearly delineated in livestock including pigs.

Urine of pregnant sows dramatically inhibited germination and shoot growth of wheat seeds than the non pregnant sows and control. Therefore, it can be concluded that seed germination inhibition is a useful technique to detect pregnancy in pigs as a simple, non-invasive, user friendly, economical and farmer's door step technique. A high concentration of abscisic acid is found in urine of pregnant cattle which maintain seed dormancy. Even though the seed germination test is highly reliable but the duration of test itself is a constraint in appraising the rural community for adoption. So, further investigation not only aimed at isolation and identification of the active inhibitory principle excreted in urine of pregnant pigs but also at improving the procedures to reduce the duration of the test to yield quick results, is highly need of today.

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