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INNOVATIVE PREGNANCY DIAGNOSIS IN HEIFERS: COMBINING SEED GERMINATION TESTS WITH URINALYSIS

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ABSTRACT

This studies assesses the seed germination test as a non-invasive, fairly priced technique for heifer pregnancy detection. 16 pregnant and sixteen non-pregnant heifers from Jam Nawaz Ali's Katohar Dairy Farm were selected at random for the study. Dilution ratios (1:6 and 1:16) with barley (Hordeum vulgare) and sorghum (Sorghum bicolor) seeds have been used to acquire and examine urine samples. The findings showed that the urine of pregnant heifers extensively suppressed each seed types' germination and shortened branch length (0.05), with barley exhibiting extra constant suppression during the route of the pregnancy. The urine study, which revealed sizable variations in ph, protein content, and color among the pregnant and non-pregnant heifers (16 pregnant and 16 non pregnant), further showed the approach's viability. The effects indicate that the seed germination test, particularly while paired with a urinalysis, may show to be an powerful diagnostic tool for cow pregnancy, offering small-scale farmers an affordable, seven-hundred rupees opportunity. For a wider range of livestock management applications, further research is vital to improve and verify the method across many cattle breeds and different livestock species

INTRODUCTION

Conventional methods of Verifying pregnancy in cattle through Invasive Diagnostic methods are costly and regularly want for veterinary knowledge. Then again, non-invasive techniques rely on breeders staring at physiological changes in ladies; nevertheless, the correctness of those techniques is tested only after parturition. New traits provide different options.

For the purpose of identifying pregnancy in ungulates, non-invasive methods have been developed, including reproductive hormone analysis in milk, urine, and feces (Bamberg et al., 1991; Kumar et al., 2013) and trans-abdominal ultrasonography (Hunnam et al., 2009). Nevertheless, these approaches are often costly.

Traditional pregnancy identification techniques are extremely specialized, labor-intensive, and costly. They include progesterone tests in plasma or milk and urine hormone assays (e.g., oestrone sulphate, pregnanediol-3-glucuronide) (Yang et al., 2003; Dilrukshi and Perera, 2009; Volkery et al., 2002).

To address these limitations, there is a need for cost-effective, accurate, and user-friendly pregnancy diagnosis methods in livestock breeding.
Skalova et al., 2013; Fedorova et al., 2015)._
Unlike traditional pregnancy diagnosis methods, the seed germination test offers a low-cost, animal-friendly, and skill-independent alternative, utilizing urine and simple equipment (Nirmala et al., 2008). This ancient technique, known as the Punyakoti test, dates back to 2200 B.C.
In order to test seeds for germination, diluted urine is applied to them (Veena Ganesaiah, 2006). Various seeds, including Research has employed different seed types, such as mung beans, wheat, sorghum bicolor, and paddy seeds, for pregnancy detection. Notably, urine from non-pregnant women demonstrated inhibitory effects on seed germination in preliminary investigations. (Ghalioungui et al., 1963). Subsequent studies extended the test to buffalo, sheep, goats, Seeds treated with the urine of pregnant females showed decreased germination, as shown by Malnad Gidda cows (Narayana Swamy et al., 2010) and mithuns (Bos frontalis) (Perumal, 2014). According to research, abscisic acid (ABA), a crucial plant hormone controlling seed dormancy, is responsible for urine's inhibitory influence on seed germination (Dilrukshi and Perera, 2009).There is a considerable difference in ABA concentrations between cows who are pregnant and those that are not (Dilrukshi and Perera, 2009; Veena Ganesaiah, 2006). With accuracy rates of 68% on day 28 and 100% during days 35–45 of pregnancy, the test's dependability in artificially inseminated cows has been shown (Rao Krishna and Veena, 2009).
Moisture, temperature, light, nutrition, and seed storage all affect how well seeds germinate (Bowden and Ferguson, 2008). Plant development depends on adequate nutrition, especially on nitrogen and phosphorus (Bolland and Bowden, 2000; Madamba et al., 2006; Bowden and Ferguson, 2008; Gentry, 2010). The ideal pH range for soil is 5.5 to 6.5. Be aware that the exception is pulses that are sensitive to hydrogen ions (Bolland and Bowden, 2000); domestic cow urine typically has a pH of 7.8 to 8.4.
In spite of earlier study, the seed germination test in domestic purebred cattle (Bos taurus) has not been studied. The current body of knowledge about alterations in seed germination in domestic crossbred cattle is limited to the early phases of gestation.(Rine et al., 2014; Krishna and Veena, 2009). We predict that the urine of pregnant purebred Czech Fleckvieh heifers will impede the germination of wheat and mung bean seeds.
The purpose of this research is to investigate the association between urine findings and seed germination in order to assess the seed germination test as a non-invasive pregnancy detection tool in European settings.
The findings may contribute to the practical application of this diagnostic method, reducing costs for small farms.

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Materials and methods

Study Design and Animals:

sixteen heifers from Katohar Dairy Farm, sixteen of them pregnant and sixteen not pregnant , Jam Nawaz Ali, District Sanghar, were randomly selected for this study. The heifers were at least 8 months old and had reached sexual maturity before receiving their first AI, with no history of reproductive or health problems.The heifers were kept under identical conditions, including feeding systems, drinking water, and veterinary care, to minimize external variables that could influence the results. AI was performed between September and November 2023, and pregnancy was confirmed on day 35 post-AI by a veterinarian using transrectal ultrasonography. ultrasonic stand, trust probe, and ultrasound machine. As controls, there were eight non-pregnant heifers. Every heifer was given a well-balanced diet that included grain, straw, corn silage, clover haylage, and mineral supplements.

Urine Collection and Storage:

At about 8:00 in the morning, urine samples were taken after breakfast.Urine samples were obtained using the free-catch technique, according to the procedure described by Haberová et al. (2012) and Rao Krishna and Veena (2009). A telescopic rod with 600-ml plastic cups attached was used for this purpose. Throughout the course of the trial, which ran from September 2023 to June 2024, samples were taken many times from the same heifers. Urine samples were taken from pregnant heifers every two weeks until day 147 of the pregnancy,

at which point the sampling period was increased to eight weeks throughout the duration of the pregnancy. The same intervals were used to sample non-pregnant heifers. In order to maintain their integrity, urine samples were processed right away after collection, chilled at 5–7°C, and utilized within 24 hours.

Analysis of urine: Under constant illumination circumstances, the color of the urine was subjectively judged as bright or dark. Utilizing Dekaphan Leuco® urine strips (Erba Lachema s.r.o., Czech Republic), urine parameters were assessed, including specific gravity, pH, leukocyte count, nitrite, proteins, glucose, ketones, urobilinogen, bilirubin, blood, and hemoglobin. A densitometer was used to determine specific gravity, and DUOTEST® pH indicator strips (pH range of 7.0–10.0; Macherey-Nagel, Karachi) were used to detect pH precisely.

Seed Germination Tests: Before being used in the seed germination studies, urine samples were chilled at 5–7°C for a maximum of 24 hours. They were then transferred to closable plastic vials (30 or 70 ml) and brought to the Sanghar Veterinary Hospital. Two ratios were used to dilute urine samples with distilled water: 1:16 (one part urine to sixteen parts water) (Dilrukshi and Perera, 2009) and 1:6 (one part urine to six parts water) (Narayana Swamy et al., 2010).

For the germination assays, seeds of two plant species—barley (Hordeum vulgare) and sorghum (Sorghum bicolor)—were used. Every urine sample was subjected to four germination tests:

Barley seeds treated with a 1:6 dilution of urine.

Barley seeds treated with a 1:16 dilution of urine.

Sorghum seeds treated with a 1:6 dilution of urine.

Sorghum seeds treated with a 1:16 dilution of urine.

For each test, 120 Seed germination was evaluated by placing 60 seeds into each of eight sterile Petri dishes, totaling 480 seeds per urine sample. Each dish received 30 ml of diluted urine. The dishes were stored in a controlled laboratory environment (25°C, natural daylight) for 7 days.

Data Collection:

From day one to day seven, the quantity of seeds that germinated was counted every day. On day seven, a ruler was used to measure the shoot lengths. Urine samples from pregnant and non-pregnant heifers were examined for seed germination rates and shoot lengths.

Information and evaluation:

We divided the pregnancy time into five phases: 25-75 days, 75-150 days, 150-200 days, 201-250 days, and above 250 days. We next examined the quantity of germinated seeds and shoot length between pregnant and non-pregnant heifers. Every pregnancy phase's seed germination findings were compared to those of females who were not pregnant.

The effects of urine color, specific gravity, pH, urobilinogen levels, and protein presence on seed germination in non-pregnant mice were investigated using urinalysis data. The StatisticaCz 12 program was used to analyze the data (StatSoft, Inc., 2013).

Preliminary analysis revealed that the seed germination data was not normally distributed (Shapiro-Wilk test, $P < 0.01$). Therefore, non-parametric Kruskal-Wallis testing was used, and P-values were separately compared. Because of the size of the dataset, shoot length analysis used ANOVA together with Tukey's post hoc test.

The link between specific gravity, pH, and the number of germinated seeds was evaluated using Spearman's rank correlation. The Kruskal-Wallis test looked at protein presence, whereas the Mann-Whitney U test assessed the effects of urine color and urobilinogen presence. For all statistical tests, a significance level of $P < 0.05$ was used.

Results

A total of 256 urine samples yeilded 22376 germinated seed

Pregnant heifers provided 124 urine samples, whereas non-pregnant heifers provided 132 samples, resulting in 22,376 germinated seeds counted and measured. Notably, both barley and In the non-pregnant heifer urine

diluted 1:16 compared to 1:6, sorghum seeds sprouted considerably higher ($P < 0.05$) on all germination test days.

When non-pregnant heifer urine was diluted, sorghum seeds showed much greater ($P < 0.0001$) germination rates than barley seeds.

How many of the seeds germinated?

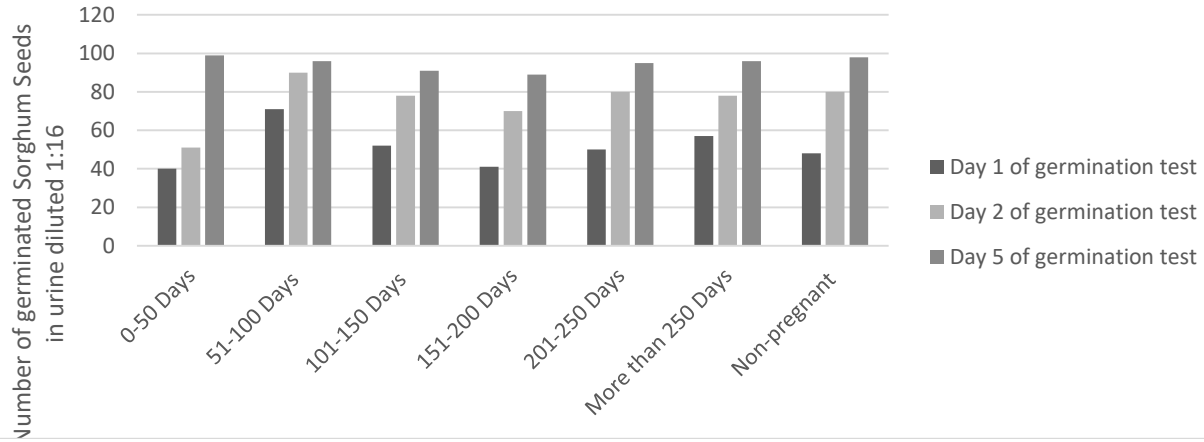
When comparing non-pregnant heifers to those at 101-150 days of pregnancy (DoP) on the first testing day, the germination of barley seeds was initially lower ($P < 0.05$ and $P < 0.0001$, respectively) in both urine dilutions (1:6 and 1:16). On the other hand, non-pregnant heifers had a substantially higher quantity of germinated seeds ($P < 0.01$) from days 2 to 5 compared to animals at 151–200 DoP. Refer to Figures 1 and 2 for comprehensive findings.

All five testing days saw a substantial ($P < 0.01$) increase in the germination of sorghum seeds in urine diluted 1:6 in non-pregnant heifers as compared to those at 101-150 days of pregnancy (DoP) (Figure 3).

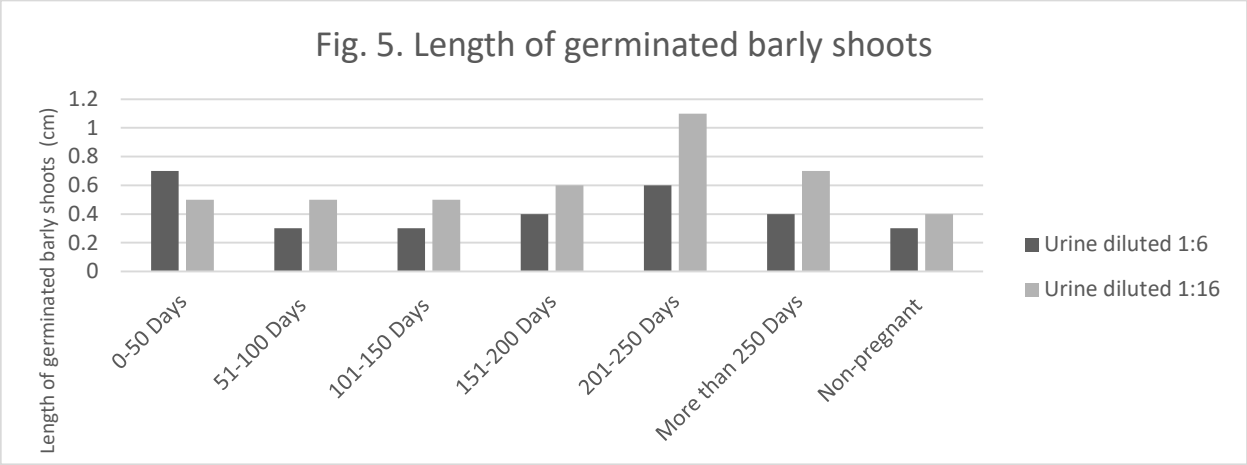
Moreover, non-pregnant heifers exhibited higher seed germination ($P < 0.05$) on day 5 compared to heifers pregnant for over 250 days.

In contrast, sorghum seeds treated with 1:16 diluted urine showed increased germination in non-pregnant females (Figure 4). Notably, a significantly lower number of Day 1 results revealed significantly enhanced germination of sorghum seeds in non-pregnant heifers versus those at 151-200 DoP ($P < 0.0001$). Thereafter, non-pregnant heifers maintained higher germination rates ($P < 0.05$) compared to pregnant heifers at 51-100 DoP throughout the remaining test period.DoP and 101–150 DoP.

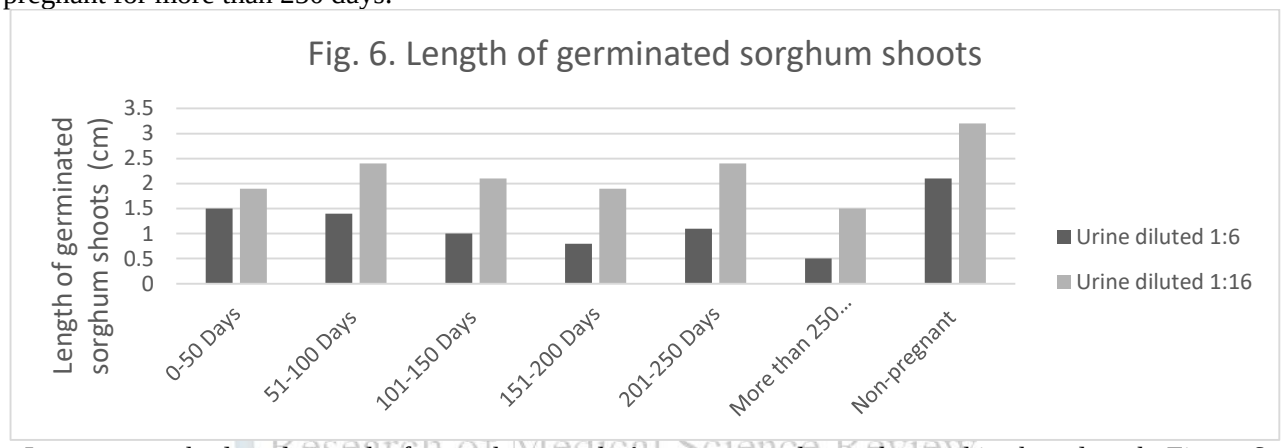
Fig. 4. Number of germinated Sorghum Seeds in urine diluted 1:16



Length of Shoots:



Regardless of urine dilution, pregnant heifers' pee substantially ($P < 0.0001$) shortened the length of sprouted barley shoots. Figure 5 shows the biggest variation between heifers that are not pregnant and those who are pregnant for more than 250 days.



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In contrast to barley, the results for sorghum seeds A reverse trend was observed in shoot length. Figure 6 presents the results, indicating that shoots germinated in non-pregnant heifers urine exhibited [insert specific characteristics].

Generally speaking, heifers' urine had longer particles than pregnant heifers' did. But the only noteworthy variation ($P < 0.01$) was seen for sorghum germinated in 1:6 diluted urine. Germinated seed shoot length varied between heifers that were not pregnant and those that were at 150–200 DoP. Furthermore, urine dilution (1:16) revealed pregnancy-stage-specific variations in shoot length, with statistically significant differences observed (see Figure 6 for specifics).

Urinalysis:

There was a noteworthy difference ($P < 0.0002$) in the urine color of pregnant and non-pregnant heifers, with the former showing darker pee (72.97%) than the latter (27.03%). Pregnant heifers showed considerably higher frequencies ($P < 0.0002$) of increased urobilinogen levels and proteins. When urine pH values were tested using DUOTEST strips, pregnant heifers' values (8.80 ± 0.02) were substantially higher ($P < 0.0002$) than those of non-pregnant heifers (8.67 ± 0.02).

While the specific gravity of non-pregnant heifers (1.002 ± 0.0002) was greater than that of pregnant heifers (1.002 ± 0.0002) according to Dekaphan Leuco strips, the difference did not reach statistical significance ($P = 0.8034$). Proteins had a significant impact on barley germination on days 3 ($P < 0.04$), 4 ($P < 0.05$), and 5 ($P < 0.01$) of the germination test, with protein-free samples showing more shoots.

In urine samples that were diluted 1:16 and had negative protein values, there were substantially more barley shoots ($P < 0.05$) on day 5. During all five days, there was a substantial increase in the number of barley shoots produced by light-colored urine diluted 1:6 and 1:16 ($P < 0.05$). In urine diluted 1:6 ($P < 0.05$), uribilinogen had a deleterious effect on barley germination. Between pH and germinated barley seeds, there were mild negative correlations ($r = -0.34$ to -0.24), and from days 3 to 5, there were weak positive correlations ($r = 0.18$ to 0.23) between specific gravity and germinated barley seeds.

Urobilinogen, proteins, or specific gravity in 1:6 diluted urine did not alter the germination of sorghum seeds. On the other hand, germination was highly dependent on urine color, with lighter-colored pee from days 2 to 5 showing greater germination of seeds ($P < 0.01$). Sorghum seeds were diluted 1:16 in urine, and no discernible effects were seen**.**

Discussion

The findings of this study offer significant data at the potential use of the seed germination test as an inexpensive, non-invasive method for identifying pregnancy in domestic cattle that are purebred, mainly in heifers of the Czech Fleckvieh breed. This study presents an amazing substitute for more intrusive and high priced diagnostic methods through supporting past studies that showed the inhibitory outcomes of pregnant ladies's urine on seed germination.

Being pregnant detection and seed germination:

Constant with other studies (Nirmala et al., 2008; Veena Ganesaiah, 2006; Narayana Swamy et al., 2010), the findings validate the inhibitory consequences of pregnant heifer urine on seed germination. Greater specifically, while uncovered to the urine of pregnant heifers, the germination fees and shoot lengths of each barley and sorghum seeds reduced. This inhibition is most likely the end result of pregnancy-associated modifications in urine composition, which may had been impacted via materials like abscisic acid (ABA), which has been proven to have an effect on seed dormancy (Dilrukshi and Perera, 2009).

Interestingly, the degree of inhibition modified according to the level of pregnancy and urine dilution. decreased germination and branch development have been repeatedly seen in barley seeds, mainly in the urine of pregnant heifers that have been nearing the end in their gestation. Conversely, the outcomes from sorghum seeds were extra inconsistent. This discrepancy ought to end result from versions in a seed's susceptibility to sure hormones or other biochemical alterations within the urine all through pregnancy. Differences in the germination desires and environmental tolerances of barley and sorghum, which include pH and nutrient ranges, can also potentially contribute to the range in responses between the 2 vegetation (Bowden and Ferguson, 2008; Bolland and Bowden, 2000).

The effect of Urinalysis on Seed Germination:

Additional help for the capacity use of the seed germination test in pregnancy detection comes from the outcomes of the urinalysis. Urobilinogen levels, urine coloration, and protein content had been discovered to vary considerably between pregnant and non-pregnant heifers. The physiological changes connected to being pregnant are possibly meditated inside the darker urine, greater protein content, and extended urobilinogen stages visible in pregnant heifers. Those results are steady with earlier studies (Veena Ganesaiah, 2006; Dilrukshi and Perera, 2009) that reviews comparable urine changes during being pregnant.

There have been also discovered correlations between the precise gravity, pH, and germination of seeds in urine. extra alkaline and much less dense urine may be less suppressive to seed germination, as proven through the weak negative link between pH and germination in barley seeds and the positive correlation among precise gravity and germination. those observations are critical for improving the accuracy and dependability of the seed germination test.

Consequences and ability uses:

In line with this study, the seed germination check may be a useful non-invasive method for determining a heifer's pregnancy, specifically in small-scale agricultural operations where access to sophisticated diagnostic system can be limited. This technique may also reduce the financial pressure on farms via providing a fee-effective opportunity and preserving animal welfare through eschewing intrusive techniques.

To improve the circumstances under which the seed germination test is executed, further investigation is important. This involves deciding on suitable seed types, figuring out the best urine dilution ratios, and figuring out the ideal time to collect samples. It would also be helpful to affirm the test's wider relevance if it had been increased to encompass animals which are crossbred and other cattle breeds. To increase diagnostic accuracy, destiny studies should investigate the possibility of integrating the seed germination check with urinalysis.

In conclusion

This study gives compelling evidence that the seed germination check is a low-cost, non-invasive approach for identifying if a heifer is pregnant. Our findings reveal that the urine of pregnant heifers notably reduces branch duration and seed germination in each barley and sorghum seeds when in comparison to the urine of non-pregnant heifers. These results persevered at numerous factors inside the being pregnant, suggesting that the technique is reliable at unique tiers of gestation.

The noteworthy variations stated in parameters related to urinalysis, together with urine color, presence of protein, and ph, bolster the possibility of the use of seed germination as a diagnostic resource. Those results mean that the inhibitory consequences on seed germination are probably a reflection of the changes in urine composition during pregnancy, maybe as a result of sure hormones or different substances located within the urine.

The seed germination test may be a beneficial supplement to the current panel of pregnancy diagnostic strategies, specifically while paired with urinalysis. For small-scale farms, wherein simplicity and economic system are vital elements, it has special blessings. This examine establishes the muse for investigating the take a look at's usefulness in many farm animals breeds and different livestock species, even though further studies is required to improve and standardize it for wider use. Using this technique can also finally result in lower charges and extra easily accessible pregnancy testing for farmers, improving productivity and animal control.

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