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SEED GERMINATION TEST AS A POTENTIAL PREGNANCY DIAGNOSIS METHOD FOR DOMESTIC CATTLE

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Abstract

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The timely diagnosis of pregnancy is of paramount importance in cattle management and non-invasive diagnostic methods may help to improve the welfare of animals. In the present study, we evaluated the seed germination pregnancy test combined with urinalysis. Urine samples were obtained from 20 non-pregnant Czech Fleckvieh heifers and from 20 artificially inseminated heifers during six phases of pregnancy: 0–50 days, 51–100 days, 101–150 days, 151–200 days, 201-250 days, and over 250 days. The seed germination test was conducted in all animals using mung beans (*Vigna radiata)* and wheat seeds *(Triticum spelta).* A total of 143 and 150 urine samples from pregnant and non-pregnant control heifers, respectively, were processed, and 37,218 germinated seeds were evaluated. The number of germinated mung beans and wheat seeds were counted over five consecutive days, and the shoot lengths were measured on day 5. On day 1 of the test, there was a higher number of germinated mung beans in urine samples obtained during the first half of pregnancy. The urine of heifers in the later stages of pregnancy showed a reduced number of germinated mung beans ($P < 0.01$). A negative influence of pregnancy on shoot length was also only noticeable in mung beans ($P < 0.0001$). On the other hand, the shortest shoots were measured in wheat seeds kept in the urine of non-pregnant heifers. Urine pH was higher in pregnant heifers than in non-pregnant heifers ($P < 0.0001$) but germination of wheat seeds was not significantly ($P > 0.05$) influenced by any urinalysis parameter, as measured using diagnostic urine strips. Thus the seed germination test evaluating the shoot length of mung beans can represent a cheap and non-invasive method to distinguish pregnant from non-pregnant heifers.

Key words: heifers; urine; *Vigna radiate*; *Triticum spelta;* shoot length; urine pH
List of abbreviations: ABA – abscisic acid, AI – artificial insemination, DoP – days of pregnancy

Introduction

Several invasive techniques for pregnancy diagnosis currently practiced in modern livestock breeding are associated with high costs, and some require a veterinarian and restraining the animal in order to reach high accuracy (Hafez and Hafez, 2000; Noakes et al., 2001; Gündoğan, 2009; Purohit, 2010). On the other hand, non-invasive methods are usually dependent on subjective assessment of physiological changes in females by the breeder, with pregnancy finally confirmed by parturition. Trans-abdominal ultrasonography (Hunnam et al., 2009) and reproductive hormone analyses of faeces, urine, milk (Bamberg et al., 1991; Kumar et al., 2013) and even saliva (Sathe, 2012; Volkery et al., 2012) represent non-invasive techniques. These can be used for the detection of pregnancy in ungulates; however, their costs usually surpass their benefits. Progesterone assays in plasma or milk, and urinary hormone assays such as oestrone sul-

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phate or pregnanediol-3-glucuronide, are highly technical, time consuming and expensive (Yang et al., 2003; Dilrukshi and Perera, 2009; Volkery et al., 2012). In addition, saliva collection requires animal cooperation and special kits (Volkery et al., 2012; Skálová et al., 2013; Fedorova et al., 2015a). In contrast to the above mentioned methods, the seed germination test utilises urine and simple equipment, is low-cost and considerate to animals, and does not require any special skills or knowledge (Nirmala et al., 2008).

The first mention of the seed germination test as a method for pregnancy diagnosis is dated to the ancient Egyptian era, around 2200 B.C., called the "Punyakoti Test". This test involves treating seeds with a solution of urine diluted with water (Veena Ganesaiah, 2006). Different seeds have been used for the seed germination test, such as mung beans (Dilrukshi and Perera, 2009), wheat seeds (Rao Krishna and Veena, 2009), sorghum bicolor and paddy seeds (Veena Ganesaiah, 2006). The inhibitory effect of urine on seed germination was initially described in women that were not pregnant (Ghalioungui et al., 1963). The seed germination test was further extended to buffalo, sheep and goats using local seeds (Veena Ganesaiah, 2006), in addition to Malnad Gidda cows (Narayana Swamy et al., 2010) and mithuns (*Bos frontalis*) (Perumal, 2014). In each of these studies, germination was inhibited in seeds that had been treated with urine from pregnant females. The inhibitory effect of urine may be due to the presence of abscisic acid (ABA), a plant hormone that causes seed dormancy in nature (Dilrukshi and Perera, 2009). ABA has been detected in the urine of pregnant cows at different concentrations to non-pregnant cows (Dilrukshi and Perera, 2009; Veena Ganesaiah, 2006). This difference was described by Veena Ganesaiah (2006) as 170.62 nmol/ml in the urine of non-pregnant cows compared to 74.46 nmol/ml in the pregnant ones'.

The reliability of the seed germination test has been determined in artificially inseminated cows as 68% on day 28 and 100% between days 35 and 45 of pregnancy (Rao Krishna and Veena, 2009).

Seed germination is in general affected by different factors such as moisture, temperature, daylight, nutrition and seed storage (Bowden and Ferguson, 2008). Adequate nutrition is essential for plant growth. The most important soil nutrients for both wheat seeds and mung beans are nitrogen and phosphorus (Bolland and Bowden, 2000; Madamba et al., 2006; Bowden and Ferguson, 2008; Gentry, 2010). The most suitable soil pH for many crops is in the range of 5.5–6.5, except for some pulses that are sensitive to hydrogen ions (Bolland and Bowden, 2000).

In healthy domestic cattle, pH of urine is 7.8–8.4 and specific gravity is in the range of 1.030–1.045 (Hofírek and Němeček, 2009). The urine of healthy cows does not contain protein, glucose, ketones, bilirubin, blood or leukocytes, and only contains a low concentration of urobilinogen (Hofírek and Němeček, 2009; Kim et al., 2010; Sink and Weinstein, 2012).

Seed germination has previously been tested in diluted urine from pregnant and non-pregnant females of several different animal breeds. However, to the best of our knowledge, no studies have reported results for heifers of pure-bred domestic cattle (*Bos taurus*). Surprisingly, the results of seed germination tests in different species have not ever been supplemented with urinalysis. Furthermore, information regarding changes in seed germination in the urine of crossbred domestic cattle are limited to the first weeks of pregnancy (Rao Krishna and Veena, 2009; Rine et al., 2014), or have only been studied at three-month intervals for cow pregnancy (Dilrukshi and Perera, 2009).

We hypothesised that germination of wheat and mung bean seeds will be inhibited by urine of pregnant pure-bred Czech Fleckvieh heifers. The aim of this study was to examine the seed germination test as a method of pregnancy diagnosis in Europe, and evaluate how the results of urinalysis can affect seed germination. Results of the seed germination test and urinalysis may lead to further application of this non-invasive diagnostic method in practice. Moreover, the use of this seed germination test can reduce the cost of pregnancy diagnosis in small farms.

Materials and Methods

Twenty pregnant and twenty non-pregnant heifers from a dairy farm Vendolí, in the Czech Republic, were randomly selected for the study. The heifers were kept under the same conditions, feeding system and veterinary treatments. All heifers were sexually mature and at least 8 months old before the first artificial insemination (AI), and were free of any reproductive or other health disorders. The heifers (n = 20) were artificially inseminated between January and March 2013, and pregnancy was confirmed from day 28 after AI (at the earliest) by a veterinarian using transrectal ultrasonography. Non-pregnant heifers (n = 20) were used as controls. All heifers were fed with mixed feed consisting of clover haylage, corn silage, straw, grain and mineral supplements.

The urine of pregnant heifers, as well as the urine of non-pregnant heifers was collected after morning feeding around 07:00. Samples were obtained during spontaneous urination using the free-catch method into 500-ml plastic cups that were fastened to a telescopic rod (Rao Krishna and Veena,

2009; Haberová et al., 2012). Urine samples were obtained repeatedly from the same females from both groups during the observation period, which lasted from April until November 2013. The urine of pregnant heifers was initially collected at two-week intervals until day 147 of pregnancy, after which the sampling interval was prolonged to 6 weeks for the second half of pregnancy. Collection of urine from non-pregnant heifers was carried out at the same time intervals as the pregnant heifers.

The samples for urinalysis were processed immediately after collection. Urine colour was assessed subjectively as either light or dark in colour. The urine colour of all samples was assessed in the same location at the same time after sampling had been completed. The following characteristics were indicated using Dekaphan Leuco® urine strips (Erba Lachema s.r.o., Czech Republic): specific gravity, pH, presence of leucocytes, nitrite, proteins, glucose, ketones, urobilinogen, bilirubin, blood, and haemoglobin. The urine density was also measured by densitometer, together with the actual temperature of urine, so that the specific gravity could also be calculated. A more accurate pH was given by DUOTEST® pH indicator strips (range pH 7.0–10.0; Macherey-Nagel GmbH & Co. KG, Germany). For the seed germination tests, urine was poured into closable plastic vials (volume 20 or 60 ml) then transported to the laboratory. Urine samples were refrigerated between 5–7°C before the seed experiment was run, for a maximum of 24 hours.

Each urine sample, collected from one animal during one sampling point, was diluted with distilled water into two dilutions: one part urine and four parts water (1:4 urine:water) (Narayana Swamy et al., 2010), and one part urine and 14 parts water (1:14 urine:water) (Dilrukshi and Perera, 2009). Seeds of two plant species were used for the test, wheat (*Triticum spelta*) and mung beans (*Vigna radiata*), with four seed germination tests performed for every urine sample: (1) mung beans treated with 1:4 dilution of urine; (2) mung beans treated with 1:14 dilution of urine; (3) wheat seeds treated with 1:4 dilution of urine; and (4) mung beans treated with 1:14 dilution of urine. For every urine sample and treatment, 100 seeds were divided into two sterile closable Petri dishes (50 seeds in each) and diluted urine samples (20 ml) were applied to the dishes. A total of eight Petri dishes (400 seeds) were used for each urine sample. The prepared germination tests were kept under laboratory conditions for 5 days at a constant room temperature of around 25°C under natural daylight.

The number of germinated mung bean and wheat seeds was determined from day 1 to 5 of the germination test, always at the same time of day. On day 5, the length of shoots was measured using a ruler.

Data analysis

Both the number of germinated seeds and the length of shoots were compared in pregnant and non-pregnant heifers. The period of pregnancy was divided into six phases: 0–50 days, 51–100 days, 101–150 days, 151–200 days, 201–250 days, and more than 250 days. The seed germination results for these periods of pregnancy were compared to the results of non-pregnant females. The results of urinalysis were compared between pregnant and non-pregnant heifers. The influence of the results of urinalysis on seed germination, especially the effects of urine colour, presence of proteins, specific gravity, pH and urobilinogen, was also analysed for control non-pregnant animals.

Data were analysed using StatisticaCz 12 software (StatSoft, Inc., 2013). As the data for the number of germinated seeds did not show a normal distribution (Shapiro–Wilk test, $P < 0.01$), the data were statistically evaluated using the nonparametric Kruskal-Wallis test with multiple comparison of P-values. The length of shoots was statistically evaluated using ANOVA, followed by Tukey's post hoc test, due to the large dataset. The influence of specific gravity and pH on the number of germinated seeds was tested using Spearman's rank correlation. The influence of urine colour and the presence of urobilinogen were tested using Mann-Whitney U test, and the presence of protein using Kruskal-Wallis test. The significance level was accepted at $P < 0.05$.

Results

In total, 143 urine samples from pregnant heifers and 150 urine samples from non-pregnant heifers were obtained. A total of 37218 germinated seeds were counted and measured. In general, significantly ($P < 0.05$) more seeds of both types germinated in urine from non-pregnant heifers diluted 1:14 compared to 1:4 for all days of the germination tests. Wheat germinated significantly ($P < 0.0001$) less frequently than mung beans in diluted non-pregnant heifer urine.

Number of germinated seeds

For both urine dilutions, the number of germinated mung bean seeds was significantly lower in non-pregnant heifers than in heifers at 51–100 days of pregnancy (DoP) on day 1 of the germination test ($P < 0.05$ and $P < 0.0001$ in urine diluted 1:4 and 1:14, respectively). From day 3 to 5 of the tests with both urine dilutions, the number of germinated seeds were significantly higher ($P < 0.01$) in the non-pregnant heifers than in animals at 101–150 DoP. For detailed results see Figures 1 and 2.

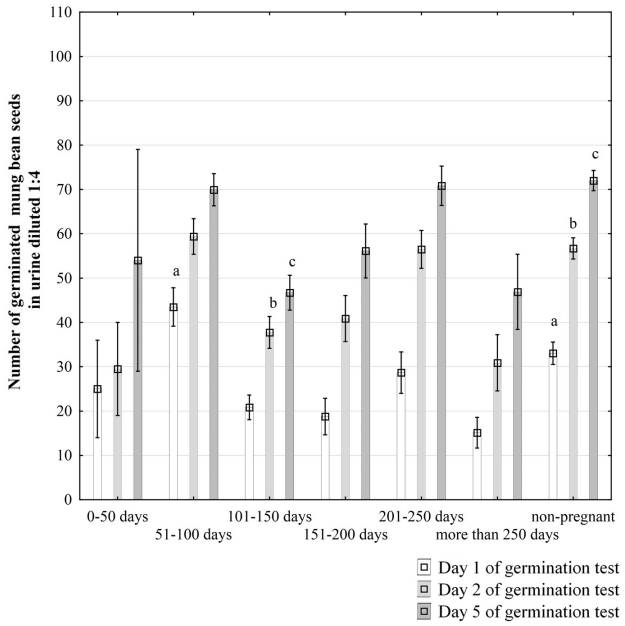

Fig. 1. Number of germinated mung bean seeds in urine diluted 1:4

Results are presented as mean ± SE. The same letters indicate significant differences between the results for heifers in the selected pregnancy period and non-pregnant heifers ($P < 0.0001$ for c; $P < 0.05$ for a, b)

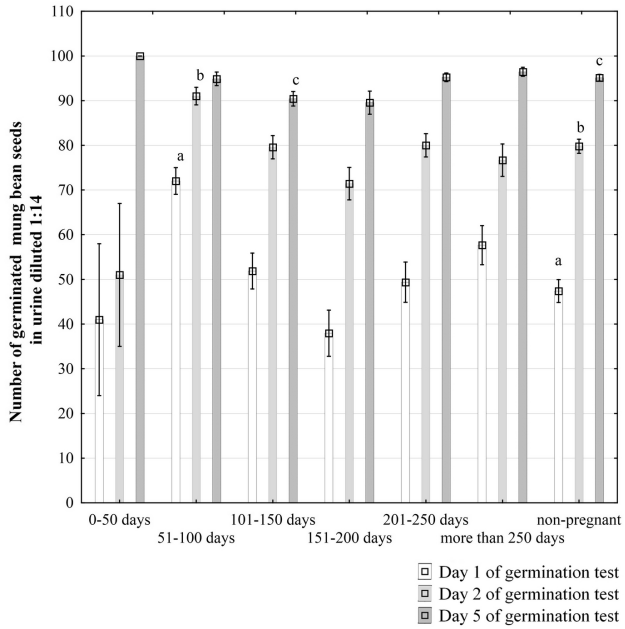

Fig. 2. Number of germinated mung bean seeds in urine diluted 1:14

Results are presented as mean ± SE. The same letters indicate significant differences between the results for heifers in the selected pregnancy period and non-pregnant heifers ($P < 0.0001$ for a, b, c)

The number of wheat seeds that germinated in urine diluted 1:4 was significantly higher ($P < 0.01$) in non-pregnant heifers than in heifers at 51–100 DoP for all five days of the germination test (see Figure 3). Moreover, a significantly higher number of seeds ($P < 0.05$) germinated in the urine of non-pregnant heifers on day 5 of the germination test than in heifers that had been pregnant for more than 250 days. On the other hand, wheat seeds treated with urine diluted 1:14 showed reduced germination in the urine of non-pregnant females (see Figure 4). A significantly lower number of germinated wheat seeds ($P < 0.0001$) were found for the urine of non-pregnant heifers than that of heifers at 151–200 DoP on day 1 of the germination test. From day 2 until the end of the germination test, a significantly lower number of germinated seeds ($P < 0.05$) were observed in non-pregnant heifers than in pregnant heifers at 101–150 DoP and 151–200 DoP.

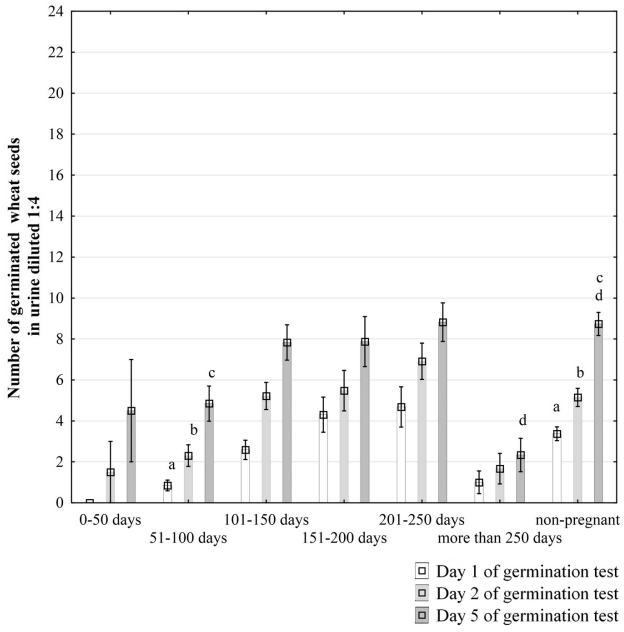

Fig. 3. Number of germinated wheat seeds in urine diluted 1:4

Results are presented as mean ± SE. The same letters indicate significant differences between the results for heifers in the selected pregnancy period and non-pregnant heifers ($P < 0.001$ for a, b, c; $P < 0.05$ for d)

Length of shoots

We showed that the urine of pregnant heifers in each phase of pregnancy significantly ($P < 0.0001$) reduced the length of germinated mung bean shoots, regardless of urine dilution. As shown in Figure 5, the largest difference was found between non-pregnant heifers and heifers that had been pregnant for more than 250 days.

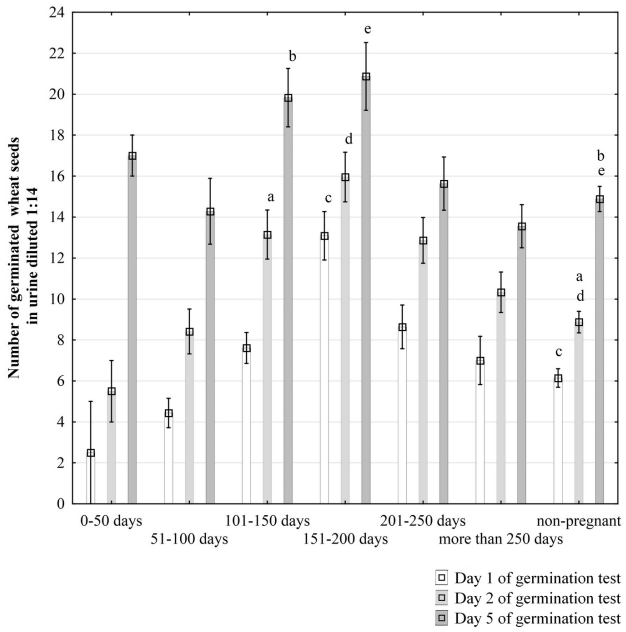

Fig. 4. Number of germinated wheat seeds in urine diluted 1:14

Results are presented as mean ± SE. The same letters indicate significant differences between the results of heifers in the selected pregnancy period and non-pregnant heifers ($P < 0.001$ for a, c; $P < 0.01$ for d; $P < 0.05$ for b, e)

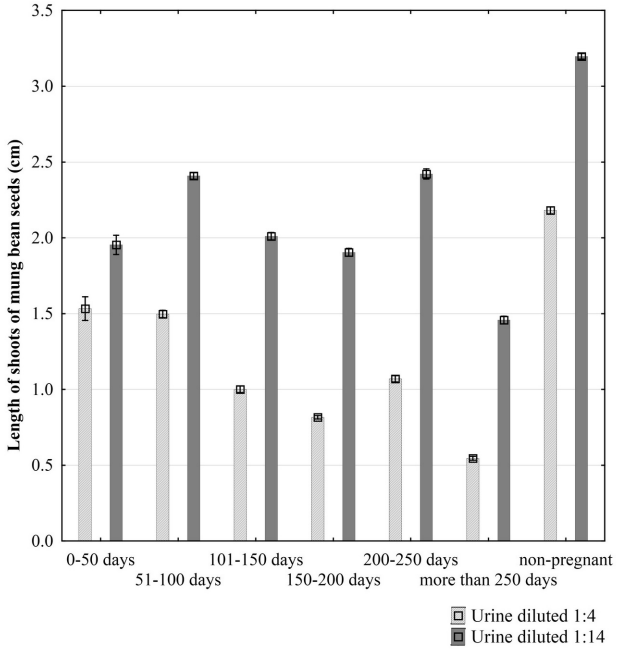

Fig. 5. Length of germinated mung bean shoots

Results are presented as mean ± SE. Results for heifers during all pregnancy periods were significantly different from non-pregnant heifers ($P < 0.0001$)

In contrast to that observed for mung beans, the results for wheat seeds showed an opposite trend for shoot length. As shown in Figure 6, the shoots that germinated in the urine of non-pregnant heifers were usually shorter than those in the urine of pregnant heifers. However, the only significant difference ($P < 0.01$) for wheat germinated in urine diluted 1:4 was found between non-pregnant heifers and heifers at 200–250 DoP. The length of shoots germinated in urine diluted 1:14 differed for several pregnancy periods, see Figure 6 for details.

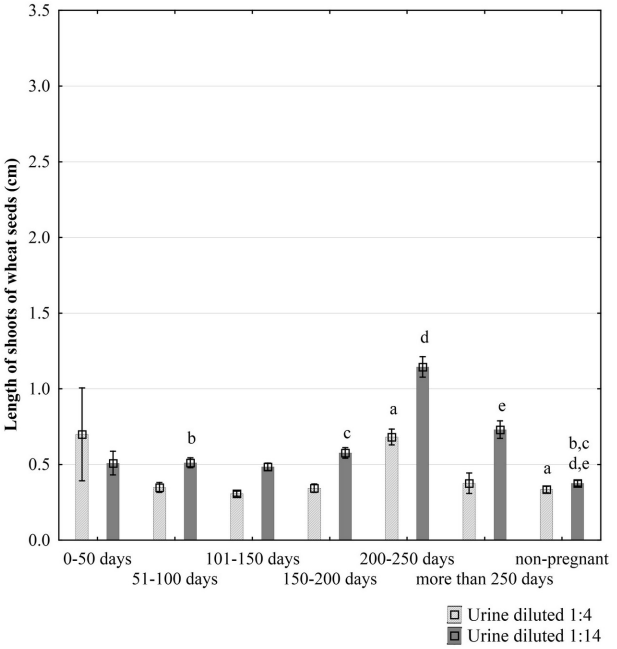

Fig. 6. Length of germinated wheat shoots

Results are presented as mean ± SE. The same letters indicate significant differences between the results of heifers in the selected pregnancy period and non-pregnant heifers ($P < 0.0001$ for a, d, e; $P < 0.001$ for c; $P < 0.05$ for b)

Urinalysis

The urinalysis comparison between pregnant and non-pregnant heifers showed a significantly higher occurrence ($P < 0.0001$) of dark-coloured urine in pregnant than in non-pregnant heifers (72.97% vs. 27.03%, respectively). The presence of proteins and increased level of urobilinogen were detected significantly ($P < 0.0001$) more often in pregnant than non-pregnant heifers. Significantly higher urine pH values ($P < 0.0001$), as determined by DUOTEST® strips, were found for pregnant compared to non-pregnant heifers (8.90 ± 0.02 vs. 8.77 ± 0.02, respectively). The specific gravity, as determined by Dekaphan Leuco® strips, was significantly higher ($P < 0.05$) in non-pregnant heifers than

in pregnant ones (1.003 ± 0.0003 vs. 1.002 ± 0.0003, respectively). However, there was no significant difference in the calculated specific gravity between pregnant and non-pregnant heifers ($P = 0.8034$).

The germination of mung beans treated with urine diluted 1:4 was significantly influenced by the presence of proteins on day 3 ($P < 0.05$), day 4 ($P < 0.05$) and day 5 ($P < 0.01$) of the germination test, where a higher number of shoots was present in samples with no protein present. A significantly ($P < 0.05$) higher number of shoots was counted on day 5 of the germination test in urine samples diluted 1:14 with a negative protein reading. Significantly ($P < 0.05$) higher numbers of mung beans shoots were counted in light-coloured urine diluted 1:4 and 1:14 over each of the 5 days of the seed germination test. Regarding urobilinogen, a higher number of mung bean shoots was detected in samples with negative findings for all five days of the germination test, but only in urine diluted 1:4 ($P < 0.05$). For both urine dilutions, significant ($P < 0.05$) weak negative correlations (r from -0.34 to -0.24) were found between the number of germinated mung beans and the pH determined by DU-OTEST® strips over the five-day seed germination test, and significant ($P < 0.05$) weak positive correlations (r from 0.18 to 0.23) were detected between the specific gravity determined by Dekaphan Leuco® strips and the number of germinated mung beans from day 3 to 5.

Germination of wheat seeds treated with urine diluted 1:4 was not significantly ($P > 0.05$) influenced by the presence of proteins, urobilinogen nor the specific gravity measured by Dekaphan Leuco® strips over the five-day germination test. In contrast, germination was influenced by urine colour, with significantly ($P < 0.01$) more wheat seeds found to germinate in light-coloured than dark-coloured urine from day 2 to 5 of the test. The number of germinated wheat seeds kept in urine diluted 1:14 was not significantly ($P > 0.05$) influenced by any other parameter tested in the urinalysis.

Discussion

Similar to other studies that have been carried out in other species (Veena and Narendranath, 1993; Perumal, 2014; Fedorova et al., 2015 b; Kubátová et al., 2016), the free-catch method of urine sampling, performed in the morning after feeding, was considered to be a suitable way to non-invasively obtain urine from heifers. Contrary to the findings of Perumal (2014), the seed germination test was practicable without the use of blotting paper, and seeds germinated well when placed directly in diluted urine in closable Petri dishes.

Similarly to the findings of Kubátová and Fedorova (2016), mung beans demonstrated better germination at the 1:14 dilution. In our study, the inhibitory effect of pregnancy was noticed at the end (day 5) of the germination test. This finding is in contrast to that of Dilrukshi and Perera (2009), who observed that fewer mung beans germinated in the urine of pregnant cows over a two-day germination test, using the same urine dilutions as the current study (1:4 and 1:14) and cows that were in similar phases of pregnancy. A similar result was obtained by Rao Krishna and Veena (2009), who described a higher germination rate of mung beans, kept in diluted urine (1:4) from non-pregnant females after two days. Such differences could be due to exposure to different periods of sunshine. The total amount of annual sunshine in Southern India is more than 2100 hours, whereas there are only 1668 hours in Prague (Czech Republic) (Osborn, 2016). Our findings for the germination of wheat seeds kept in urine diluted 1:4 are in agreement with Veena and Narendranath (1993), Krishna Rao and Veena (2009) and Rao Krishna and Veena (2009), who all reported poorer germination in the test with urine from pregnant females after two days. In accordance with Narayana Swamy et al. (2010) and Rine et al. (2014), who demonstrated an inhibitory effect of pregnancy in the wheat germination test in urine diluted 1:4 after three days. According to our results, the test with wheat seeds kept in urine diluted 1:14 can be used to identify non-pregnant females. This is incompatible with the results of Veena Ganesaiah (2006), who noticed suppressed germination after five days in samples treated with urine from pregnant animals. On the other hand, these results are in agreement with a study conducted in women (Ghalioungui et al., 1963), where no growth of wheat seeds of two varieties (baladi and hindi) occurred in the urine of non-pregnant women.

Our results for the length of mung bean shoots are consistent with Dilrukshi and Perera (2009), who observed a suppression effect of pregnancy on germination after five days. On the other hand, the average length of shoots in their study, in both pregnant and non-pregnant cows, was longer than that observed in our study. This could be due to the higher number of seeds in one Petri dish used in the current study, which would exhibit higher demand on the amount of solution. While Dilrukshi and Perera (2009) used 15 ml of diluted urine for only 15 mung bean seeds, we treated 50 mung bean seeds with 20 ml of urine solution. The better germination ability observed in their trials may have also been influenced by pre-soaking the seeds before the germination test.

Our results for wheat shoot length also differ from other studies. Both Veena and Narendranath (1993) and Veena Ganesiah (2006) reported a dramatic inhibitory effect of pregnancy status on the length of wheat shoots. However, it should be noted that these authors used local varieties of wheat, which may have different seedling emergence characteristics.

According to the results of our study, mung beans were better for pregnancy status estimation than wheat seeds. This finding could be due to the good and fast seedling emergence of pulses (Madamba et al., 2006). In addition, differences in the optimal temperature for wheat and mung beans could affect the results of germination tests. According to Khamassi et al. (2013), the optimal temperature for beans is around 20°C, with 27–30°C reported to be the optimal temperature range for mung beans (Madamba et al., 2006; DAFF, 2010). Cramb et al. (2000) found that 25°C is the optimal temperature for wheat growth, whereas Lee and Herbek (2009) described an adequate temperature range for wheat germination of 12.5–25°C. Unfavourable temperature conditions may delay seed germination (Baskin and Baskin, 2001). As for floral induction, wheat requires a lower temperature, with the optimal temperature varying between 7–18°C for spring types and 0–7°C for winter types. Furthermore, wheat does not grow well under warm conditions with high humidity (Madamba et al., 2006), and the high relative humidity, due to the use of closed Petri dishes, may have influenced the results of the current study. The temperature may have also led to differences between the results of our germination test and those of other available studies. Many similar studies have been performed in India, mainly in Southern India, where the average annual temperature is between 19–34°C (Osborn, 2016). The average temperature in the laboratory was not as high, and therefore, it could be assumed that local wheat varieties from India or Egypt are more tolerant of higher temperature than wheat from more moderate zones.

Sundra et al. (2004) reported that ruminant urine has a neutral or slightly alkaline pH. Urinary pH can be influenced by composition of feed, infection, storage time, and metabolic and respiratory alkalosis (Bethard and Stokes, 2000; Sundra et al., 2004; Kume et al., 2011). The values of urine pH in our study differ from those of Veena and Narendranath (1993). The mean values of urine pH in pregnant and non-pregnant females were generally lower than those presented by Veena and Narendranath (1993) of 7.38 ± 0.40 and 7.08 ± 0.37 in pregnant and non-pregnant cows, respectively, over 3 months after calving. However, the findings of Dilrukshi and Perera (2009) do not support an influence of urine pH on seed germination, as all groups of tested cows had similar urine pH values in their study, leading the authors to conclude that the inhibitory effect on seed germination was not likely due to urine pH. A lower urinary pH in pregnant females can be caused by the presence of ABA (Veena Ganesaiah, 2006). Lower wheat seed emergence in diluted urine, as opposed to mung beans, observed in our study could be caused by higher urine pH. The optimal soil pH for normal wheat growth is 5.5–6.0 (Fageria et al., 2010) whereas mung bean seeds require slightly acid soil for adequate growth, specifically 6.3–7.2 (DAFF, 2010). Guo et al. (2009) reported a negative effect of alkaline stress on wheat germination. Therefore, it is possible that the acidic urine pH found in heifers caused the reduced germination capacity in wheat seeds. This assumption is supported by the fact that soil pH influences nutrient availability (Bolland and Bowden, 2000).

The values of specific gravity, as indicated by the Dekaphan Leuco® strips, were within the range of physiological values reported by Reece (2004) and Kim et al. (2010) in both pregnant and non-pregnant heifers. The results of urinalysis showed that the number of germinated seeds can be negatively influenced by the presence of proteins. Although the urine of healthy animals does not contain protein (Hofírek and Němeček, 2009; Sink and Weinstein, 2012), our results showed the presence of protein in the urine of pregnant females. In the present study, fewer mung beans germinated in the urine of non-pregnant heifers that showed positivity for proteins. However, according to Dekaphan Leuco®, false positive values for protein can occur in alkaline urine with a pH greater than 8 (ErbaGroup, 2012).

Conclusions

The use of free-catch urine sampling was evaluated in Czech Fleckvieh heifers, which can be performed with the animal in a standing or lying position. The results of the seed germination test showed significant differences between pregnant and non-pregnant heifers, both in the number of germinated shoots and their shoot length. Pregnancy diagnosis by counting the number of germinated mung beans is possible at both tested dilutions (1:4 and 1:14) between 101 and 150 days of pregnancy. Pregnancy inhibited the number of germinated mung beans from day 3 to 5 of the germination test. The seed germination test using wheat seeds should be practiced only with urine at a dilution 1:4, for which pregnancy inhibited germination from day 1 until the end of the test. An inhibitory effect of pregnancy on shoot length was shown in mung beans. In general, mung beans demonstrated better germination at the 1:14 dilution. The shortest shoot length of wheat seeds tested in 1:14 diluted urine was detected in non-pregnant heifers. The results of urinalysis showed that light-coloured urine is more frequently observed in non-pregnant heifers, in addition to a negative finding for the presence of proteins and normal urobilinogen values. The urine pH was higher in pregnant heifers. The influence of urinalysis on seed germination was also tested in non-pregnant heifers, where a greater number of mung beans germinated in light-coloured urine with negative findings for proteins and a physiological concentration of urobilinogen.

The shoot length of mung beans in the seed germination test can be used to distinguish pregnant from non-pregnant heifers as a simple and non-invasive method which does not require expensive laboratory equipment or chemicals.

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