

PRACTICAL EVALUATION PREGNANCY OF HEIFERS BY PUNYAKOTI TEST

Renalda Juodžentytė, Vytuolis Žilaitis

*Large Animals clinic, Veterinary academy, LSMU, Tilzes str.18, Kaunas, Lithuanian
E-mail: renaldajuodzentyte@gmail.com*

Abstract. In this study, the seeds germination inhibition technique was applied to diagnose pregnancy of Lithuanian Black and White dairy heifers. The urine samples collected from 14 heifers. The urine was diluted at the ratio of 1:4 with distilled water. In each sterile Petri dish, fifteen wheat seeds were taken on the blotting paper and 15 ml of diluted urine was added. Control test was also carried out with the addition of distilled water only to the wheat seeds. It was collected the urine samples on days 14, 24, 34 and 44 post embryo transplantation. Pregnancy diagnosis confirm with ultrasound device „Draminski animal profi 2” at 44 days after embryo transplantation. The germination rate was 15.0, 26.67, 19.27 and 9.27 per cent in pregnant heifers on days 14, 24, 34 and 44 post embryo transplantation. The shoot lengths of the germinated wheat seeds was 1.0, 0.4, 0.4, 0.5 cm in pregnant heifers on days 14, 24, 34 and 44 post embryo transplantation. Inhibition of germination and shoot growth were attained by the urine of pregnant heifers and does. In conclusion, seed germination test can be used as a simple and non-invasive method to detect pregnancy in heifers.

Keywords: Seed germination, Heifers, Shoot length, Pregnancy

Introduction. Diagnosing the pregnancy at early stage is an important requirement for successful dairying and to improve the production of farmers (Wani et al., 2003).

The seed germination (viability) inhibition technique was applied to diagnose pregnancy in cattle. It was a detailed study to determine what chemical agent in the urine affects different grain germination. In cattle urine apart from the normal urinary constituents such as urea and uric acid, a plant hormone known as Abscisic acid (ABA) has been identified. It's main effect of ABA is to maintain the dormancy of the seeds (Dilrukschi et al., 2009).

A high concentration of ABA is found in the urine of pregnant cows (170.62 nanomoles/ml of urine) as compared with that in the urine of non-pregnant cows (74.46 nanomoles/ml) (Veena et al., 2003; Hai-Hang Li et al., 2011). The presence of ABA could be one of the factors causing the observed decreased germination (Veena et al., 2003).

Wani (2003) argued that hormone metabolites excreted through urine of pregnant animal might affect the seed germination. The test performed with different concentrations of hormones - estradiol and progesterone (Wani et al., 2003).

In the world for many years, the seed germination inhibition technique was applied to diagnose pregnancy of indigenous sheep and cattle after insemination, but not embryo transplantation. Therefore, in this study we wanted to extend the research and to determine the possibility of using the seed germination inhibition technique for dairy heifers after embryo transplantation.

Objective

Evaluation of possibility to estimate the early pregnancy of heifers after embryo transfer by grain germination (Punyakoti test).

Material and methods

The research was conducted in accordance with the provisions of the Law of the Republic of Lithuania No. 1-

2271 on Protection, Keeping and Use of Animals, dated 03/10/2012 (Valstybės žinios (Official Gazette) No. 122-6126 dated 20/10/2012) and of the by-laws, Education and training purposes of animals used in storage, maintenance and conditions of use No. B1-866, dated 31/10/2012 (Valstybės žinios (Official Gazette) No. 130-6595 dated 10/11/2012).

Our research was carried out on 14 Lithuanian Black and White dairy heifers. Embryo transplantation was performed by „minitub” (Germany) instruments. It was collected the urine samples on days 14, 24, 34 and 44 post embryo transplantation. Pregnancy diagnosis confirm with ultrasound device „Draminski animal profi 2” at 44 days after embryo transplantation.

The urine samples were collected in plastic tubes between 7:00 – 8:00 in the morning and diluted at the ratio of 1:4 with distilled water. For each heifers was prepared two samples 1 –experiment - the sample (diluted urine + seeds), 2 - control (water + seeds).

In each sterile Petri dish 15 wheat seeds were taken on the blotting paper and 15 ml of diluted urine was added (1 ml urine + 14 ml distilled water) first group. Second group - control test was also carried out with the addition of water only to the wheat seeds. Samples stored under cover at room temperature, after three days, the seeds were examined for germination rate and shoot lengths. Shoot length growth in wheat seeds measurement with the ruler. Heifers pregnancy was determined by 2 signs: 1. seeds germination. 2. shoot length. We hold that heifers pregnant, if seeds were germinated 5 from 15. Pregnancy heifers shoot length were until 0,9 cm (Perumal, 2014).

Results

The investigated showed that the less seed germination determined 52.79 percent. ($P < 0.05$) in pregnant heifers group than non-pregnant and 72.65 percent. ($P < 0.05$) than water control. The less seed germination determined 19.86 percent. ($P > 0.05$) in non-pregnant group than water control. (Figure 1).

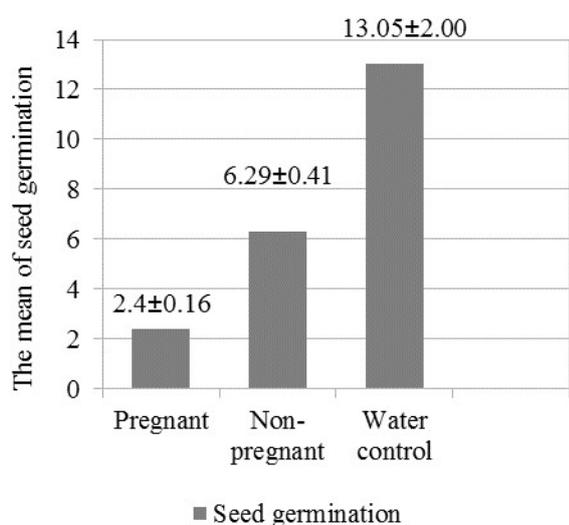


Fig. 1. Germination percentage of wheat seeds in Pregnant and Non-Pregnant heifers compared to Water control.

Its showed that the shoot lenght in pregnant heifers group is 79.34 percent. smaller ($P < 0.05$) than non-pregnant and 87.16 percent. smaller ($P < 0.05$) than water control. The the shoot lenght in non-pregnant group is 37.83 percent. smaller ($P > 0.05$) than water control. (Figure 2).

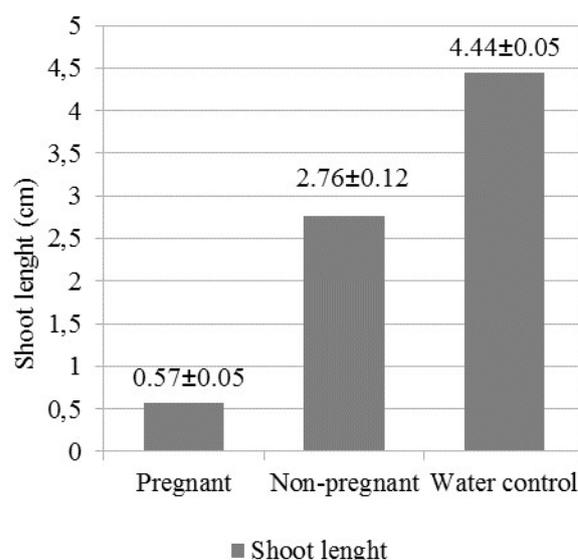


Fig. 2. Shoot lenght (cm) of wheat seeds in Pregnant and Non-Pregnant heifers compared to Water control.

The average mean of separated days showed that the less seed germination determined 22.17 percent. ($P < 0.05$) in pregnant heifers group on 14 day than pregnant heifers group on 34 day. The less seed germination determined 18.98 percent. ($P < 0.05$) in non-pregnant group on 14 days than non-pregnant group on 34 day. (Figure 3).

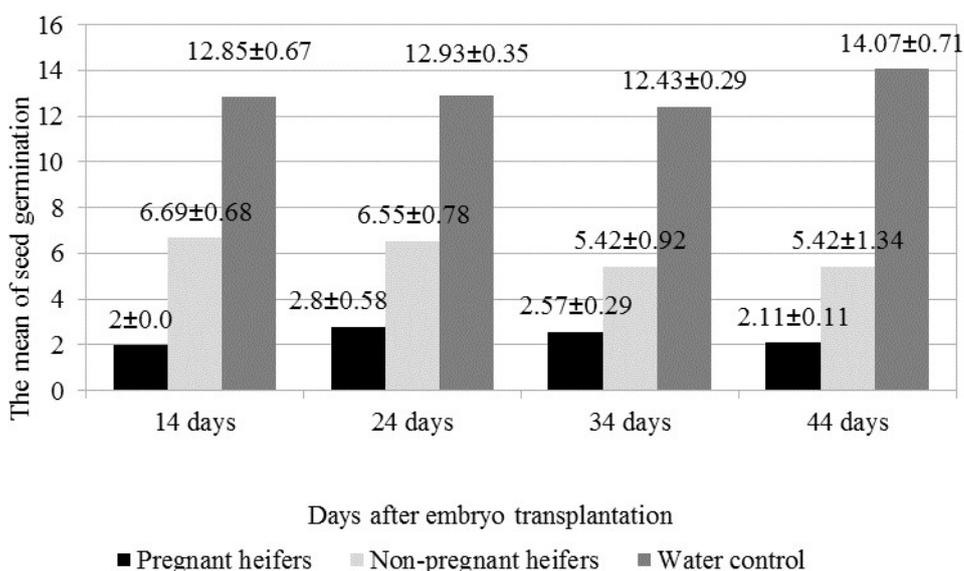


Fig. 3. Seed germination of seeds treated with water, urine of pregnant and of non-pregnant heifers.

The average mean of separated days showed that the shoot lenght in pregnant heifers group is 60.0 percent. smaller ($P < 0.05$) on 24 day than pregnant heifers group on 34 day. (Figure 4).

By shoot lenght and seed germination show the pregnancy in 34 and 44 days after embryo transplantation. The test results fully coincide with the ultrasound results. (Figure 5).

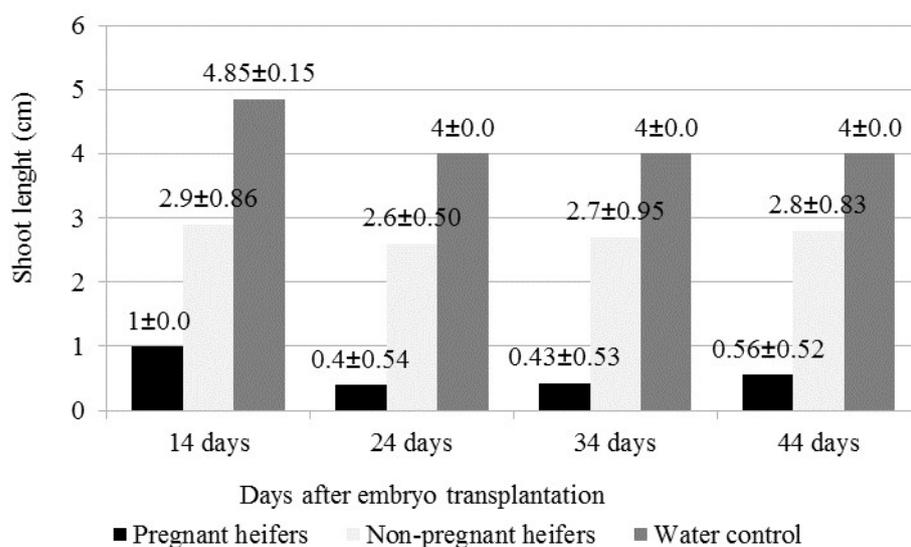


Fig. 4. Shoot length (cm) of seeds treated with water, urine of pregnant and of non-pregnant heifers.

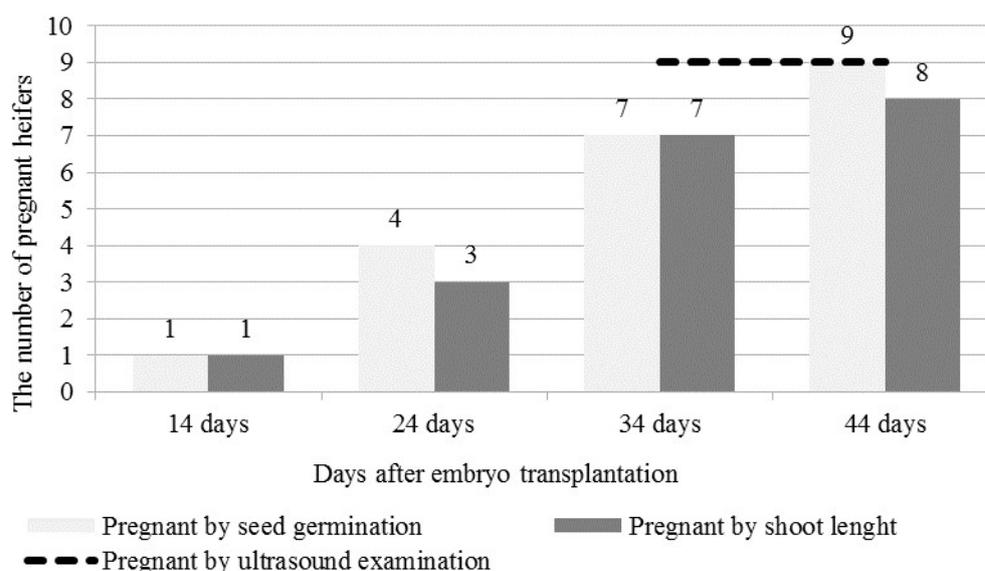


Fig. 5. The number of pregnant heifers by ultrasound examination, shoot length and seeds germination.

Discussion

Many studies established by that 14 days after insemination the percentages of seeds germination average were 59.80 and 62.47 in pregnant and non-pregnant heifers, respectively, and 82.97 in control. The mean shoot length of the germinated wheat seeds was 2.69 cm and 4.92 cm in pregnant and non-pregnant heifers, respectively an 7.73 cm in control groups (Rao Krishna, Veena, 2009).

We found that 14 days after embryo transplantation the percentages of seeds germination were 15.00 and 73.33 in pregnant and non-pregnant heifers. The mean shoot length of the germinated seeds was 1.0 cm and 2.9 cm in pregnant and non-pregnant heifers.

By M. Z. Rine (2014) 24 days after insemination the percentages of seeds germination were 45.88 and 60.27 in pregnant and non-pregnant cows, respectively, and 59.20 in control. The mean shoot length of the germinated wheat seeds was 2.84 cm and 3.40 cm in pregnant and non-pregnant cows, respectively an 3.54 cm in control groups.

By our results 24 days after embryo transplantation the percentages of seeds germination were 26.67 and 80.00 in pregnant and non-pregnant heifers. The mean shoot length of the germinated wheat seeds was 0.4 cm and 2.6 cm in pregnant and non-pregnant heifers.

By M. Z. Rine (2014) 34 days after insemination the percentages of seeds germination were 43.47 in pregnant

and 59.13 non – pregnant cows. 44 days after insemination the percentages of seeds germination were 34.69 in pregnant and 59.40 non – pregnant cows. According to M. Narayana Swamy (2010) 44 days after insemination the mean germination inhibition percentage was 23.54, 72.10 and 78.52, respectively, in pregnant, non-pregnant and water control groups. Detection of mean shoot length of the germinated wheat seeds on early pregnancy in cows was 0.95, 3.62 and 5.54 cm in pregnant, non – pregnant and water control groups. P. Perumal (2014), has established that after insemination the percentages of wheat germination were 19.00 in pregnant and 75.98 non – pregnant cows. Detection of mean shoot length of the germinated wheat seeds on early pregnancy in cows was 0.53, 3.67 and 5.91 cm in pregnant, non – pregnant and water control groups.

By ours results 34 and 44 days after embryo transplantation the percentages of wheat germination were 14.27 and 72.96 in pregnant and non-pregnant heifers. The mean shoot length of the germinated wheat seeds was 0.49 cm and 2.75 cm in pregnant and non-pregnant heifers.

Conclusion

All groups grain germination averages: pregnant heifers (17.55 per cent.), non-pregnant (74.81 per cent.), and water control (92.96 per cent.). The mean shoot length of the germinated wheat seeds was 0.59 cm and 2.75 cm in pregnant and non-pregnant heifers, respectively and 4.21 cm in control groups. According to grain germination heifers pregnancy accurately determined 44 days after embryo transplantation.

Punyakoti test can identify early pregnancy of 44 days after embryo transplantation. The test results fully coincide with the ultrasound results.

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