

## Urinary reproductive hormones influence seed germination within diluted urine of heifers: alternative pregnancy diagnostic method

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## 1 **SUMMARY**

Experimental non-invasive pregnancy diagnostic techniques, such as seed germination testing in diluted urine, may serve as an alternative to standardized pregnancy diagnostic methods, but scientific validation of these methods is required. This study aimed to use Czech Fleckvieh heifers to investigate the influence of urinary reproductive hormones (oestrone sulphate, 17β-oestradiol, and pregnanediol-3-glucuronide) on the germination success of seeds placed within their diluted urine, and further to verify the reliability of seed germination test for pregnancy diagnosis in this species. Mung bean and wheat seeds were germinated in two urine-water dilutions (1:4 and 1:14) for three days, using urine samples obtained from either pregnant or non-pregnant heifers. Germinated seeds were counted daily, and the shoot lengths were measured three days after placement in the urine. Levels of urinary reproductive hormones were determined using competitive heterogeneous enzyme immunoassays (EIAs). Despite the dilution rate used, final results indicated that the urine from pregnant heifers inhibited germination and growth in mung beans only. On the contrary, wheat germination rate was higher within the urine from pregnant females on days 1 and 2, when using a 1:14 dilution rate. For both seed species, correlations between urinary hormone profiles and seed germination parameters were found, depending on the day of the experiment and the rate of dilution used. The shoot lengths of mung bean seeds were significantly (P < 0.05) and negatively correlated with the profiles of all hormones analysed from the urine samples. Thus, urinary reproductive hormones influence seed germination and growth rate of wheat and mung beans, indicating the potential for this technique to be used for pregnancy determination in heifers.