

## Early Pregnancy Diagnosis Using Transrectal Colour Doppler Ultrasonography and Serum ISG15 mRNA assay in Dairy Cows

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**Introduction:** Early pregnancy diagnosis is an important factor in the management of reproduction in dairy cows. However, tools for accurate detection of non-pregnancy or pregnancy status within first 21 days of insemination are limited. Colour Doppler ultrasonography, which allows detailed assessment of the uterus, ovarian follicles and corpora lutea, has been utilized as a successful technique for bovine pregnancy detection. Interferons are involved in maternal recognition of pregnancy and interferon-stimulated genes (ISG) are up-regulated during pregnancy. The present study examined the suitability of two novel techniques based on these concepts to diagnose pregnancy in early gestation.

**Materials and Methods:** Transrectal ultrasound examinations were performed in 16 synchronized and artificially inseminated (AI) cows at day 20 post-AI (pAI). Images of corpus luteum blood flow (CLBF) were obtained using colour ultrasound scanner (CDUSS) and the images were analysed using image analysis software ImageJ. Animals were categorized as pregnant or non-pregnant based on the results of CLBF and confirmed on day 45 pAI by per-rectal palpation. Serum was collected from the same set of animals on day 20 pAI and polymerase chain reactions (PCR) were performed to identify ISG15 mRNA using extracted RNA. Differential expressions of ISG15 in pregnant and non-pregnant cows were analysed using semi-quantitative RT-PCR.

**Results:** Significantly higher ( $p < 0.05$ ) arterial CLBF was observed in pregnant cows at day 20 pAI. The CLBF had only 60% accuracy in detecting non-pregnancy. A sensitivity of 100% was achieved in detection of pregnant animals and 60% specificity for determination of non-pregnant animals through CLBF analysis. A 100% negative predictive value (NPV) reflected the absence of false negative results. The overall accuracy of CLBF evaluation on day 20 was 75%. Semi-quantitative PCR analysis revealed a significant up-regulation in the ISG15 gene in pregnant compared to non-pregnant cows ( $p < 0.05$ ) on day 20 pAI.

**Discussion:** The main purpose of early pregnancy diagnosis is to re-breed the non-pregnant animals as early as possible to produce one calf per year. The findings of this study support non-pregnant identification (NPV=100%) at day 20 pAI using CDUSS. Primary factors resulting in false positive diagnosis could be early embryonic losses between days 20 and 45 pAI, extended oestrous cycles with delayed CL regression and short oestrous cycles with ovulation in the same ovary, rather than misdiagnosis. ISG15 mRNA is reported to be up-regulated after day 16, peak at day 20 and decline by day 32. This experiment should be extended with real-time quantitative PCR to confirm the change in gene expression.

**Conclusion:** CLBF evaluation is a reliable diagnostic tool with high sensitivity and 100% NPV for identification of non-pregnant cows. It helps in reducing open days and calving intervals substantially by allowing early resynchronization of cows. Low blood ISG15 mRNA levels on day 20 pAI may be a predictive biomarker of non-pregnancy in dairy cows.

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