

## Acupuncture increases matrix metalloproteinase activity in bovine caruncles after calving

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### Introduction

Placental release is a complex physiological process involving matrix metalloproteinase (MMP) lysis to separate cotyledon from caruncle. If MMP activity is absent or diminished placental retention can result due to lack of collagenase activity.<sup>1</sup> Acupuncture has been used as a treatment for other reproductive disorders in dairy cattle including delayed uterine involution, repeat breeding, and uterine infections.<sup>2</sup> The objectives of this study were to determine the effects of acupuncture in dairy cows on caruncular MMP activity at 0, 2, and 4 hours after calving. We hypothesized that acupuncture would increase caruncular MMP activity.

### Methods

Immediately after natural calving, cows were restrained in a stanchion. The perineum was aseptically prepared and a caruncle was obtained from the body of the uterus, and flash frozen. Caruncle collection was repeated at 2 and 4 hours after calving. Acupuncture was administered to cows (n=6) following each caruncle collection (at 0 and 2 hours after calving). Untreated controls (n=9) were kept in a stanchion for 15 minutes without any stimulation. Acupuncture was applied to points reported to relax the cervix and stimulate uterine contractions: bladder 31, 32, and 34, Baihui, Guanyuanshu, and governing vessel-1. Frozen caruncles (0.5 g) were homogenized in solubilization buffer and centrifuged. The supernatant was collected and the protein concentration was determined. Zymography was performed by electrophoresis on 8% polyacrylamide and 0.6% gelatin. Gels were incubated in a 2.5% Triton X-100 renaturing buffer and stained with Coomassie blue R-250. Gels were immersed in destaining solution to develop lytic zones (bands) indicating enzymatic activity. Enzymatic activity was quantified using a digital camera and true 16-bit data capture system (Gel Logic 2200 Pro, Bio-Techniques). Average band intensities (indicating enzyme activity) for each molecular mass category between treatment groups and time points were compared using Student's t-test. Significance was defined as  $p < 0.05$ .

### Results

Five distinct calcium-dependent gelatinases (234, 143, 117, 59, and 49 kDa) were found in the bovine caruncles at 0, 2, and 4 hours after calving. There was a trend for acupuncture to decrease the activity of the 234 kDa gelatinase at 4 hours after calving compared to controls ( $p = 0.08$ ). The average enzyme activities did not differ between acupuncture and controls for the 143, 117, and 59 kDa gelatinases. Enzyme activity of the 49 kDa gelatinase significantly increased from 2 to 4 hours after calving in acupuncture-treated cows and significantly decreased from 0 to 2 hours after calving in control cows.

### Discussion

The lowest molecular mass gelatinase (49 kDa) corresponds to the active form of MMP type 2. These results are supported by previous research in our laboratory that showed that acupuncture increased tissue concentrations of MMP type 2 from 2 to 4 hours after calving.<sup>3</sup>

**Keywords:** Caruncle, cow, matrix metalloproteinase, placentome, retained placenta

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### References

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