## 10.1071/RD24163

Reproduction, Fertility and Development

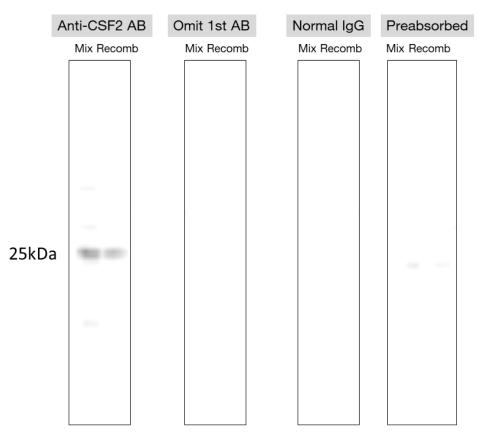
## **Supplementary Material**

Lower expression of colony-stimulating factor 2, an embryokine, in the endometrial epithelium of old cows

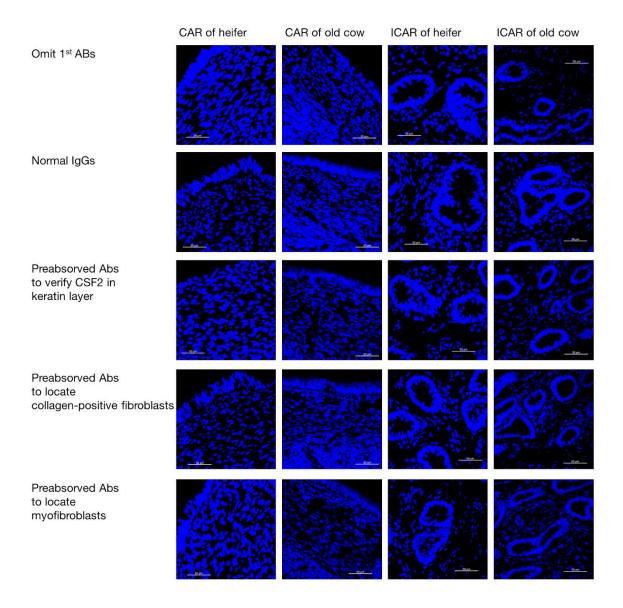
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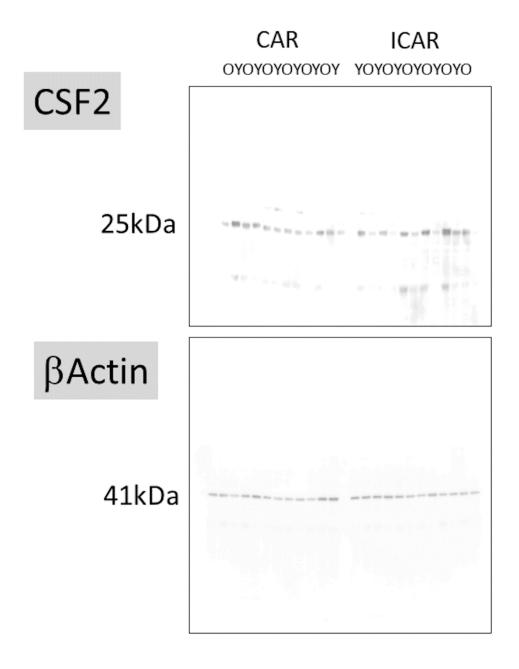
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Supplementary Figure S1. Comparison of western blot images to verify the specificity of the signal. After electrophoresis of the bovine endometrium protein mixture (Mix) and recombinant bovine CSF2 (Recomb), membranes were cut into four pieces and treated with either anti-CSF2 antibody, no antibody (omitted negative control), normal rabbit IgG, or the anti-CSF2 antibody pre-absorbed with the antigen peptide.



Supplementary Figure S2. Comparison of merged immunohistochemistry images to verify the specificity of the signal, using no antibody (omitted negative control), normal rabbit IgG, or the anti-CSF2 antibody pre-absorbed with the antigen peptide. These methods were used to verify CSF2 in the keratin layer, locate collagen-positive fibroblasts, or locate myofibroblasts in the CAR or ICAR of heifers or old cows. Scale bars represent  $50~\mu m$ .



Supplementary Figure S3. A photo of a whole western blot for CSF2 was used for the statistical analyses. O: Old group. Y: Young group