Total protein lysates were prepared, and the amounts of the phosphorylated forms of Smad1/5/8 were determined by western blot analysis. Interestingly, treatment with 500 ng/ml BMP-6 induced phosphorylation of Smad. The BMP-6 induced phosphorylation was high at the earliest time point tested (15 minutes), and remained high for at least 48 hours (Figure 5). (PMC-1134658)