Effects of Recombinant Bovine Somatotropin Administration at Breeding on the Cow, Conceptus and Subsequent Offspring Performance of Beef Cattle

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Synopsis
The objective of this study was to determine if administration of recombinant bovine somatotropin (bST) to beef females at breeding has the potential to increase fertility, conceptus development, and subsequent offspring performance. Injection of 325 mg of bST around the time of breeding increased concentration of insulin-like growth factor-1 (IGF-1) and increased expression of myxovirus-2 on d 21 of suckled beef cows. However, it failed to alter fetal development and postnatal offspring performance.

Summary
To determine the effects of administration of a low dose of slow-release bST (Posilac, Elanco, Greenville, IN) on hormone concentration and conceptus development, a total of 190 suckled beef cows were exposed to the 7-d CO-Synch+CIDR fixed-time AI (TAI) protocol. Cows were blocked by days postpartum, BCS, breed, and randomly assigned to receive one of the following treatments: 1) two injections of 325 mg bST, one at TAI and a second injection 14 d after TAI (D-bST, n=40); 2) one injection of 325 mg bST at TAI and a placebo (saline) injection 14 d after TAI (TAI-bST, n=48); 3) a placebo injection at TAI and one injection of 325 mg bST 14 d later (14D-bST n=49); and 4) two injections of placebo, one at TAI and a second injection 14 d after TAI (Ctrl, n=53). Pregnancy was determined via transrectal ultrasonography 35 d after TAI and conceptus development was assessed by measuring crown to rump length (CRL) on d 35 and crown to nose length (CNL) on d 65 after TAI. Blood samples were collected on d 0, 7, 14, 21, 35 and 65 relative to TAI to determine concentrations of IGF-1, and on d 18 and 21 for isolation of peripheral blood leukocytes (PBL) and mRNA expression of interferon stimulated genes. Plasma concentrations of pregnancy-specific protein B (PSPB) were also assessed on d 35 and 65 after TAI. Individual calf birth weight and sex were determined at birth. Liver biopsies were performed in a subset of calves at 8 ± 3 d of age, and expression of target genes related to the somatotropic axis were determined. There were no differences (P=0.77) among treatments on pregnancy to TAI (48.7±0.5%). Administration of bST at TAI increased (P<0.0001) plasma concentration of IGF-1 on d 7, 14, and 21. However, CRL and CNL (0.48±0.02 in d 35 and 0.67±0.001 in d 65, respectively) did not differ (P=0.23) among treatments. Concentration of PSPB did not differ (P=0.18) among treatments and between days (P=0.30; 2.69±0.11 ng/mL), and gestation length (282±9 d) did not differ (P=0.49) among treatments. However, mRNA expression of myxovirus-2 on d 21 differed (P=0.05) among treatments. Cows receiving an injection of bST at TAI had a greater fold increase in MX-2 mRNA expression compared to cows receiving an injection of bST at TAI and 14 d later. Injection of bST did not alter calf liver mRNA expression of insulin-like growth factor-1 (P=0.81), IGF-2 (P=0.29), and IGFBP-3 (P=0.66). However, injection of bST at TAI tended (P=0.06) to increase calf liver mRNA expression of IGFR-1 compared to the calves born to cows in other treatments. In addition, calf birth weight was similar (P=0.52) among treatments. We conclude that administration of 325 mg bST during the time of TAI to suckled beef cows enhanced concentrations of IGF-1, but failed to improve pregnancy rates, fetal size, PSPB concentrations, and had no effect on calf birth weight.
**Introduction**

Developmental programming, also termed “fetal programming”, is the concept that perturbations during critical prenatal development stages may have lasting impacts on postnatal growth and adult function (Godfrey, 1998). Growth and development of the embryo and fetus are complex biological events influenced by genetic, epigenetic, maternal maturity, as well as environmental and other factors. These factors affect the size and functional capacity of the placenta, uteroplacental transfer of nutrients and oxygen from mother to fetus, conceptus nutrient availability, the fetal endocrine milieu, and metabolic pathways. The somatotropic axis and its major components growth hormone (GH) and IGF-1 are an essential constituent of multiple systems controlling growth and reproduction (LeRoith et al., 2001). In addition, the somatotropic axis plays a key role on embryonic and fetal development by acting directly on the oocyte, endometrium, placenta, and embryo.

Early exposure of ovine embryos to increased concentrations of GH and IGF-1 has been linked to enhanced prenatal development and increased postnatal growth, with alterations in the somatotropic axis (Costine et al., 2005; Koch et al., 2010). In addition, strategies focusing on supplementation of GH and IGF-1, through administration of recombinant bovine somatotropin (bST), during the time of embryonic implantation and maternal recognition of pregnancy have been successful on enhancing conceptus development and increasing fertility of dairy cattle (Ribeiro et al., 2013). However, little information exists on the effects of bST treatment on beef females at breeding and in subsequent offspring performance.

Therefore, we hypothesized that administration of bST to beef females at breeding has the potential to increase fertility, conceptus development and subsequent offspring performance.

**Materials and Methods**

A total of 190 multiparous suckled beef cows composed of Angus, Brangus, and Braford breeds were enrolled in the experiment. All cows were subjected to the 7-day CO-Synch + CIDR estrus synchronization protocol. In brief, cows received a 100-μg injection of GnRH (2 mL Factrel; Zoetis Animal Health) at CIDR (1.38 g P4; Zoetis Animal Health) insertion [d -10] with a 25-mg injection of PGF$_2$α (5 mL Lutalyse; Zoetis Animal Health) at CIDR removal [d -3], followed by an injection of 100-μg GnRH and TAI [d 0] at 66 h after CIDR removal. Cows were blocked by days postpartum, body condition score (BCS), breed and randomly assigned to receive one of the following treatments (Figure 1): 1) two injections of placebo (1 mL of 0.9% saline), one at TAI and a second injection 14 d after TAI (CTRL, n=53); 2) two injections of 325 mg bST (Posilac, Elanco Animal Health, Greenville, IN, USA), one at TAI and a second injection 14 d after TAI (2bST, n=40); 3) one injection of 325 mg bST at TAI and a placebo injection 14 d after TAI (TAIbST, n=48); and 4) a placebo injection at TAI and one injection of 325 mg bST 14 d later (d14bST n=49).

Blood samples from each cow were collected on d 0, 7, 14, 18, 21, 35 and 65. Concentrations of total IGF-1 were determined by a commercial ELISA kit (Quantikine ELISA Human IGF1 Immunoassay, R&D Systems, Inc., Minneapolis, MN, USA). Concentrations of PSPB were analyzed using a commercially available quantitative ELISA assay (BioPRYN, BioTracking LLC, Moscow, ID). Blood samples were collected on days 18 and 21 for isolation of peripheral blood leukocytes (PBL) and mRNA expression of interferon stimulated genes (ISG).

Pregnancy status was determined by transrectal ultrasonography on d 35 and 65 with an Ibex portable ultrasound equipped with a linear 5 MHz multi-frequency transducer (E.I. Medical Imaging, Loveland, CO, USA). Embryo crown to rump length (CRL) was measured on d 35 and fetal crown to nose length (CNL) was measured on d 65 post-TAI. Images were measured twice using an image capturing software (ImageJ, National Institute of Health, Bethesda, Maryland, USA) for later measurements and final CRL and CNL values were calculated as the mean between the two measurements.
Calf body weight was determined within 12 hr of birth and every 30 days until weaning using a scale (TRU-TEST, Mineral Wells, TX, USA). Cow-calf pairs were maintained together on pasture from birth until weaning with ad libitum access to bahiagrass hay, bahiagrass pasture, and water. A subset of 40 pregnant cows of Angus and Brangus breeds and 24 calves (12 males and 12 females; not necessarily born from the subset of 40 cows) equally distributed among treatments were selected for analysis of mRNA expression of target genes. Liver biopsies were performed in a subset of calves at 8±3 d of age, and expression of target genes related to the somatotropic axis were determined.

Extraction of RNA was conducted according to recommendations of the RNA-extraction kit manufacturer (PureLink RNA Mini Kit, Invitrogen, Carlsbad, CA). A total of seven genes were investigated, including five target genes, myxovirus 2 (MX2), 2′-5′-oligoadenylate synthetase 1 (OAS1), insulin-like growth factor 1 (IGF1), insulin-like growth factor 2 (IGF2), insulin-like growth factor 1 receptor (IGF1R), and insulin-like growth factor binding protein 3 (IGFBP3); and two reference genes, cyclophilin-1 and ribosomal protein S9 (RPS9).

Cows were blocked by breed and stratified by days post-partum, and body condition score (BCS) and randomly assigned to treatments. All data was analyzed as a randomized block design using the SAS statistical package (SAS Inst. Inc., Cary, NC) with animal as the experimental unit. Concentrations of IGF-1, P4, PSPB, and the embryo/fetal measurements CRL and CNL were analyzed as repeated measures using the MIXED procedure. The models included the effects of day, treatment and its interactions, and the random effect of animal. Pregnancy rates were analyzed using the GLIMMIX procedure. The model included the effects of treatment, days post-partum, BCS, breed and its interactions. Calf body weight was analyzed as repeated measures using the MIXED procedure. The model included the effects of sex, treatment and its interactions.

**Results**

Injection of 325 mg of bST to suckled beef cows increased plasma concentration of IGF-1 (Figure 2). Plasma concentrations of IGF-1 were similar (P=0.92) among treatments at TAI on d 0 (64.8±2.6 ng/mL). Cows receiving an injection of bST at TAI on d 0 had greater (P<0.0001) plasma concentrations of IGF-1 on d 7 and on d 14. Accordingly, cows receiving an injection of bST at TAI on d 14 had greater (P<0.0001) plasma concentrations of IGF-1 on d 21. Concentrations of progesterone (P4) of pregnant cows increased (P<0.0001) after TAI (0.25, 2.39, 5.37, 4.78 and 5.09±0.26 ng/mL, for d 0, 7, 14, 21 and 35, respectively). However, injection of 325 mg of bST did not alter concentration of P4 (P=0.49) among treatments and no treatment × day interaction was detected (P=0.26). Concentrations of PSPB did not change (P=0.30) between d 35 (2.76±0.109 ng/mL) and 65 (2.62±0.110 ng/mL) post TAI, and did not differ among treatments (P=0.55).

Pregnancy rates to TAI were determined by ultrasonography on d 35 post TAI and was similar among treatments (45.3, 45.0, 51.0 and 54.2 for CTRL, 2bST, TAIbST and d14DbST, respectively) and gestation length (282±17 d) was also similar (P=0.33) among treatments (Table 1). Fetal size was determined by ultrasonography on d 35 and 65 post TAI (Table 1) and was not altered by injection of bST. Crown to rump length on d 35 was similar (0.48±0.02 in; P=0.61) among treatments. In addition, crown to nose length on d 65 (0.67±0.001 in) did not differ (P=0.23) among treatments.

Expression of ISG of pregnant cows was determined on d 18 and 21 post TAI. Relative mRNA expression of OAS-1 was similar among treatments on d 18 (1.8, 1.0, 1.2 and 0.9-fold increase for TAIbST, d14DbST, 2bST and CTRL, respectively; P=0.22) and on d 21 (1.6, 1.0, 2.2 and 0.9-fold increase for TAIbST, d14bST, 2bST and CTRL, respectively; P=0.23). In addition, relative mRNA expression of MX-2 (Figure 3) was similar among treatments on d 18 (1.2, 1.3, 0.9 and 1.5-fold increase for TAIbST, d14bST, 2bST and CTRL, respectively;
However, mRNA expression of MX-2 on d 21 differed \( (P=0.05) \) among treatments. Cows receiving an injection of bST at TAI had a greater fold increase in MX-2 mRNA expression compared to cows receiving an injection of bST at TAI and 14 d later (2.0 and 0.8, for TAIbST and 2bST, respectively), while d14bST and CTRL were intermediate (1.2 and 0.9, respectively).

In our study, bST injection had no effect on calf performance from birth to weaning (Table 1). Birth weight was similar \( (P=0.65) \) among treatments (79.4, 72.9, 73.4 and 75.4±4.47 kg for TAIbST, d14bST, 2bST and CTRL, respectively). Furthermore, body weight did not differ \( (P>0.10) \) among treatments at 30, 60, 90, 120 and 150±17 d of age (163.8±5.59, 231.0±8.44, 303.3±9.36, 376.3±8.18, and 450.4±9.08 kg, respectively).

Injection of bST did not alter calf liver mRNA expression (Figure 4) of IGF-1 \( (P=0.81) \), IGF-2 \( (P=0.29) \), and IGFBP-3 \( (P=0.66) \). However, injection of bST at TAI tended \( (P=0.06) \) to increase calf liver mRNA expression of IGFR-1 compared to the calves born to cows in other treatments (3.1-fold compared to 1.0, 1.2, and 1.5-fold for TAIbST, 2bST, d14bST, and CTRL, respectively).

**Conclusion**

In the present study, injection of 325 mg of bST around the time of breeding increased concentration of IGF-1 and increased expression of MX-2 on d 21 of suckled beef cows. However, it failed to alter fetal development and postnatal offspring performance. Further investigation is needed on the dose and timing of administration of bST and its effects on fetal development and postnatal offspring performance of beef cattle.

**Acknowledgements**

Sincere appreciation is expressed to P. Moriel, A. Ealy, S. Johnson, P. Folsom, M. Foran, O. Helms, D. Jones, C. Nowell, and D. Thomas for their assistance with data collection and laboratory analysis. The authors thank Zoetis Animal Health (Florham Park, NY) for their donation of PGF \(_{2\alpha}\) (Lutalyse), GnRH (Fertagyl), and CIDR inserts (CIDR EAZI-Breed).

**Literature Cited**

Table 1. Fertility, gestation, fetal development and calf performance measurements from suckled beef cows previously treated with bST.

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<th>P-value</th>
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$^1$-bST, injection of 325 mg of bST on d 0 and 14; TAI-bST, injection of 325 mg of bST on d 0 and placebo injection on d 14; 14D-bST, placebo injection on d 0 and injection of 325 mg of bST on day 14; CTRL, placebo injection on d 0 and 14.

$^2$CRL – crown-to-rump length; CNL – crown-to-nose length.
Figure 1. Experiment outline and schematic of treatments. All cows were estrous synchronized using a 7-Day CO-Synch + CIDR protocol where cows received an injection of GnRH on d -10 and CIDR was inserted, followed by injection of PGF2α (PGF) and CIDR removal on d -3, followed by injection of GnRH and fixed-timed AI (TAI) on d 0. Individual body condition scores (BCS; 1=thin to 9=obese) were assigned on d -10. Blood samples (B) were collected on d 0, 7, 14, 18, 21, 35 and 65. Pregnancy diagnosis and fetal measurements were performed by ultrasonography (US) on d 35 and 65. Treatments: CTRL, placebo injection on d 0 and 14; 2bST, injection of 325 mg of bST on d 0 and 14; TAIbST, injection of 325 mg of bST on d 0 and placebo injection on d 14; d14bST, placebo injection on d 0 and injection of 325 mg of bST on day 14.
Figure 2. Concentrations of insulin-like growth factor 1 (IGF-1) of beef cows on days relative to fixed-timed AI (TAI) by treatment. Treatments: CTRL, placebo injection on d 0 and 14; 2bST, injection of 325 mg of bST on d 0 and 14; TAIbST, injection of 325 mg of bST on d 0 and placebo injection on d 14; d14bST, placebo injection on d 0 and injection of 325 mg of bST on day 14. \(^{a,b}P<0.01.\)

Figure 3. Expression of Myxovirus 2 (MX2) mRNA on d 18 and 21 of gestation on suckled beef cows treated with bST. Treatments: CTRL, placebo injection on d 0 and 14; 2bST, injection of 325 mg of bST on d 0 and 14; TAIbST, injection of 325 mg of bST on d 0 and placebo injection on d 14; d14bST, placebo injection on d 0 and injection of 325 mg of bST on day 14. \(^{a,b}P=0.05.\)
Figure 4. Expression of hepatic insulin-like growth factor 1 (IGF1), insulin-like growth factor 2 (IGF2), insulin-like growth factor 1 receptor (IGF1R), and insulin-like binding protein 3 (IGFBP3) mRNA of calves born to suckled beef cows treated with bST. Treatments: CTRL, placebo injection on d 0 and 14; 2bST, injection of 325 mg of bST on d 0 and 14; TAIbST, injection of 325 mg of bST on d 0 and placebo injection on d 14; d14bST, placebo injection on d 0 and injection of 325 mg of bST on day 14. *b P=0.06.