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Superovulatory response and embryo development in ewes treated with two doses of bovine somatotropin

J.M. Carrera-Chávez[a,1], J. Hernández-Cerón[b], M.A. López-Carlos[a], R.R. Lozano-Domínguez[a], F. Molinar[c,d], F.G. Echavarría-Cháirez[a], R. Bañuelos-Valenzuela[a], C.F. Aréchiga-Flores[a,*]

[a] Unidad Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Zacatecas, Carretera Panamericana Zacatecas-Fresnillo Km 31.5, 98500 El Cordovel Enrique Estrada, Zacatecas, Mexico
[b] Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Av. Universidad 3000, 04510 México, DF, Mexico
[c] United States Department of Agriculture, 11940 Don Haskins Dr. Suite E-3, El Paso, TX 79936, USA
[d] Departamento de Ciencias Veterinarias, Universidad Autónoma de Ciudad Juárez, Henry Dunant s/n Zona Pronaf, 32315 Ciudad Juárez, Chihuahua, Mexico

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ABSTRACT

This study evaluated whether the administration of 50 and 100 mg bovine somatotropin (bST) at the start of synchronization and at the time of natural mating in ewes improves the ovulation rate, embryonic development and pregnancy rate of transferred embryos. Forty-eight donors were assigned to three treatments: the bST-100 treatment ($n = 15$) received 100 mg bST at the start of synchronization and at natural mating, the bST-50 treatment ($n = 15$) received 50 mg bST on the same schedule as the previous group, and the control ($n = 18$) did not receive any bST. Two embryos were transferred to each recipient ($n = 121$): 35 received embryos from bST-100; 50 received embryos from bST-50, and 36 received embryos from the control. The superovulatory rate, percentage of recovered structures, cleavage rate, percentage of transferable embryos, embryo quality and development and pregnancy rate were analyzed using the GENMOD procedure of SAS. The number of corpora lutea and the cell number were analyzed using the GLM procedure of SAS. The insulin and IGF-1 concentrations were analyzed with ANOVA for repeated measures. The bST application did not affect the superovulatory rate, number of corpora lutea and recovered structures ($P > 0.05$). The numbers of transferable embryos and embryos reaching the blastocyst were higher ($P \leq 0.01$) in the bST-50 ($96.4 \pm 3.6\%$ and $69.0 \pm 7.8\%$) than the bST-100 ($93.0 \pm 4.5\%$ and $27.2 \pm 38.9\%$) and control ($87.7 \pm 5.4\%$ and $50.4 \pm 6.4\%$) groups. The insulin and IGF-1 concentrations were higher ($P < 0.05$) in the bST-treated groups, but the insulin concentration was higher ($P < 0.05$) in the bST-100 group than in the bST-50 group. The pregnancy rate was similar ($P = 0.21$) in ewes receiving embryos from the two treatments [bST-50, (70.0%); bST-100, (62.5%), and control, (56.6%)]. The administration of 50 mg bST at the start of synchronization and at natural mating in superovulated ewes was concluded to enhance the proportion and development of transferable embryos. However, bST did not affect the pregnancy rate of transferred embryos.

* Corresponding author. Tel.: +52 47 89 85 12 55; fax: +52 47 89 85 02 02.
E-mail addresses: jose.carrera@uacj.mx (J.M. Carrera-Chávez), arechiga@uaz.edu.mx (C.F. Aréchiga-Flores).
[1] Present address of First author: Departamento de Ciencias Veterinarias, Instituto de Ciencias Biomédicas, Universidad Autónoma de Ciudad Juárez, Anillo Envolvente del Pronaf y Estocolmo s/n, Zona Pronaf 35315 Ciudad Juárez, Chihuahua, México. Tel.: +52 656 639 8800.

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1. Introduction

Multiple ovulation and embryo transfer (MOET) is a tool to maximize the progeny of high genetic merit ewes. However, the great variability in the superovulatory rate affects the efficiency of quality embryo production (Oliveira, 2011).

The exogenous administration of bovine somatotropin (bST) increases the circulating concentration of insulin and insulin-like growth factor 1 (IGF-1) in sheep (Gong et al., 1996; Joyce et al., 1998; Montero-Pardo et al., 2011). Research suggests that insulin and IGF-1 are responsible for the effects of bST on reproduction (Joyce et al., 1998; Carrillo et al., 2007; Camacho et al., 2008). These effects include an increase in the number of recruited follicles (Gong et al., 1996; Ramón et al., 1998) and an enhancement in the number and development of embryos produced (Montero-Pardo et al., 2011; Mejía et al., 2012).

Insulin and IGF-1 receptors have been detected in sheep follicles (Scaramuzzi et al., 2010). The insulin on follicular cells enhances the glucose and amino acid metabolism, stimulates cell proliferation and growth, inhibits follicular steroid secretion (Gallet et al., 2011), and modulates the function of the gonadotrophin receptor (Muñoz-Gutierrez et al., 2002; Somchit et al., 2007).

Insulin-like growth factor-1 synergizes with FSH in granulosa cells (Beg and Ginther, 2006) to improve hormonal activities, such as the secretion of follistatin, activin-A, and inhibin-A; the proliferation and differentiation of granulosa cells; the production of estradiol, and the regulation of aromatase activity (Silva et al., 2009). Overall, IGF-1 protects and promotes the maturation of the oocyte (Neira et al., 2010).

Research on the effects of bST in ruminants indicates that the hormone improves embryonic development and therefore increases the reproductive efficiency (Ribeiro et al., 2014). bST itself and the resulting IGF-1 concentration enhance the nuclear maturation rate and metabolism of pyruvate, have antiapoptotic effects during the *in vitro* development of bovine embryos (Stefanello et al., 2006), and increase the number of cells of the blastocyst (Moreira et al., 2002b; Sirisathien et al., 2003). Moreover, these hormones modulate the synthesis of $PGF_2\alpha$ synthesis (Badinga et al., 2002).

In studies of sheep, the commercially available bovine dose of bST (500 mg) is commonly divided in four equal portions of 125 mg each. Several studies have administered this dose before or at the time of mating. The results of these studies indicated an improvement in the litter size (Carrillo et al., 2007) and embryonic development in sheep (Montero-Pardo et al., 2011; Mejía et al., 2012). Moreover, another study utilizing the same dose in anestrous goats reported an increase in the pregnancy rate (Martínez et al., 2011).

However, several studies reported contradictory effects on the superovulatory response and pregnancy rate after the application of bST (Eckery et al., 1994; Hasler et al., 2003; Bilby et al., 2004; Camacho et al., 2008; Rivera et al., 2010). Other authors have suggested that this variability may be due to several factors, such as the bST dosage, physiological condition, and the resulting serum concentration of IGF-1 (Block et al., 2005; Velazquez et al., 2009; Ribeiro et al., 2014). For example, the bST treatment that favors conception rate in lactating cows (Bilby et al., 2006) decreases the fertility rate in non-lactating cows (Bilby et al., 2004). The deleterious effect of bST on the pregnancy rate in non-lactating cows may be related to the hyperstimulation of blood insulin and IGF-1 secretion (Bilby et al., 2004). In sheep, the different responses found in several studies may be attributed to the bST dosage.

The objective of this study was to evaluate whether the administration of 50 and 100 mg bovine somatotropin to superovulated ewes at the start of synchronization and at the time of natural mating improve the ovulation rate, embryonic development, and pregnancy rate of transferred embryos in sheep.

2. Materials and methods

2.1. Animals and treatments

This study was performed during the breeding season (autumn and winter) in Zacatecas, Mexico. The donors were 48 cyclic, adult Dorper ewes (3–5 years old), and the recipients were 121 cyclic, adult hair sheep ewes (Pelibuey, Blackbelly, Katahdin, Dorper and their crosses) (3–5 years old). Both the donors and recipients were housed in total confinement and fed oats and alfalfa hay, ground yellow corn, soybean meal, and minerals. All ewes had at least one parturition prior to the experiment and a body condition score between 3 and 4 on a scale of 5 (Russel et al., 1969).

The ewes were synchronized with controlled release intravaginal devices (CIDR, DEC Manufacturing, Hamilton, New Zealand), which remained in place for 12 days. Simultaneously to the application of CIDR (day 0), all ewes received an intramuscular injection of 0.05 mg/kg body weight of selenium and 0.05 mg/kg body weight of vitamin E (Mu-Se, MSD Animal Health, Mexico). After the removal of the intravaginal device, all recipients received an intramuscular injection of 250 IU of equine chorionic gonadotropin (Pregnecol, Bioniche Life Sciences Inc., Australia).

On day 0, the donors were randomly assigned to three treatments: (1) the bST-100 treatment ($n = 15$) received a subcutaneous injection of 100 mg bST (Boostin-S, MSD Animal Health, Mexico) at the start of synchronization and a second injection at natural mating; (2) the bST-50 treatment ($n = 15$) received a subcutaneous injection of 50 mg bST (Boostin-S, MSD Animal Health, Mexico) according to the same program as previous group; and (3) the control ($n = 18$) received saline solution instead of bST.

Superovulation started 10 days after the CIDR placement. Superovulation was induced with a total of 164 mg of pFSH (Folltropin-V, Bioniche, Ontario, Canada) administered as eight decreasing doses every 12 h. Two days after CIDR removal, the ewes were served by rams of proven fertility every 1 h starting at 09:00 h until each donor received five services.

Three or more CL per ewe was considered a superovulatory response (Folch et al., 2001). The embryos were collected using a semi-laparoscopic technique (Bari et al., 2000). The same experienced technician conducted all collections. The embryos were classified according