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A single dose of bovine somatotropin 5 days before the end of progestin-based estrous synchronization increases prolificacy in sheep

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Abstract

Bovine somatotropin (bST) enhances ovarian follicular and embryonic development in sheep and cattle. In the present study, the objective was to assess whether bST given 5 days before the end of progestin-based estrous synchronization improves prolificacy and lambing rate in sheep. Pelibuey ewes ($n = 92$) exhibiting estrous cycles at regular intervals received an intravaginal sponge containing 45 mg of FGA for 12 days. Five days before sponge withdrawal, ewes were treated with either 125 mg of bST sc (bST group; $n = 47$) or saline solution (control; $n = 45$). After the sponge was removed, ewes were observed for estrus and subsequently mated twice. Lambing rate and prolificacy was determined at birth. Blood samples were taken from the time of treatment until day 15 after estrus in eight ewes from the bST group and nine from the control group. Concentrations of IGF-I were determined by immunoradiometric assay and progesterone by RIA. Treatment with bST increased ($P < 0.01$) the proportion of ewes with more than one lamb (bST, 56% compared with control, 26%) and prolificacy (bST, 1.6 compared with control, 1.3). Treatment with bST increased ($P < 0.05$) the lambing rate of multiparous (bST, 92% compared with control, 67%) but not in ewes at the first time they were mated (bST, 71% compared with control, 87%; $P > 0.05$). IGF-I concentrations were greater ($P < 0.01$) in ewes treated with bST than in control ewes from 2 days after treatment. Progesterone concentrations did not vary ($P > 0.05$) between groups. It is concluded that a single dose of bST 5 days before progestin withdrawal increases lambing rate

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and prolificacy in sheep. These effects are associated with an increase in circulating concentrations of IGF-I.

Keywords: Sheep-somatotropin; Estrus; Hormonal-flushing; Lambing rate; Prolificacy

1. Introduction

Early embryonic mortality is the main cause of pregnancy failure in sheep where 20–30% of the embryos die during the first 13 days after fertilization (Nancarrow, 1994). In addition, retarded growth of embryos is associated with embryonic mortality (Barnes, 2000). In sheep, somatotropin treatment increases the proportion of embryos in advanced stages of development at the time of embryo recovery (Rosas et al., 1995) and improves the rate of transferable embryos in superovulated ewes (Folch et al., 2001). In cattle, bST improved embryonic survival and conception rate (Morales-Roura et al., 2001; Moreira et al., 2002b; Santos et al., 2004). Somatotropin treatment and its associated increase in IGF-I improve ovarian follicular development (De La Sota et al., 1993) and corpus luteum function (Lucy et al., 1994; Lucy et al., 1995) in cattle. *In vitro*, IGF-I improves bovine oocyte maturation (Lorenzo et al., 1994) and early embryonic development (Moreira et al., 2002a). In addition, IGF-I could function as an embryonic survival factor sparing preimplantation embryos from the effects of detrimental factors (Jousan et al., 2004). Therefore, a treatment with growth hormone could improve fertility and prolificacy in sheep. The present experiment was designed to test the hypothesis that increased IGF-I concentrations during preovulatory follicular and early embryonic development will improve prolificacy and lambing rate in sheep.

2. Materials and methods

2.1. Animals and treatments

The study was performed during the breeding season (August–October) in the dry-tropical state of Nayarit, México. Pelibuey ewes ($n = 92$) having estrous cycles at regular intervals varying in age were kept on pasture and balanced across treatments. Time of estrus was synchronized with an intravaginal sponge containing 45 mg of fluorogestone acetate (FGA) (Chronogest, Intervet, México), which remained in place for 12 days. On the tenth day of treatment, all ewes received an intramuscular injection of the prostaglandin analogue luprostiol (Prosolvin, Intervet, México). Five days before sponge withdrawal ewes were randomly assigned to two groups: bST group ($n = 47$) received a depot injection of 125 mg of bST sc (Lactotropina, Monsanto Comercial, México). The control group ($n = 45$) received equal amount of saline solution. From the day the sponge was removed, estrus was detected aided by a ram fitted with an apron and ewes in estrus were subsequently mated twice. Lambing rate was calculated as the percentage of ewes lambing from the total of ewes mated and the prolificacy was determined by the number of lambs born per ewe lambing. Birth weight was recorded at lambing.

2.2. Blood sampling and radioimmunoassays

Blood samples (10 mL) were taken daily using vacutainer tubes with sodium heparin (Becton Dickinson, México) from eight ewes of the bST group and nine from the control group, beginning

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at the time of bST injection until day 15 after estrus. Plasma was obtained after centrifugation at 1500 rpm/10 min and stored at −20 °C. IGF-I concentrations were quantified in duplicate (León et al., 2004) every other day in a single immunoradiometric assay (DSL-2800 kit, Diagnostic Systems Laboratories Inc., Houston, TX) with an intra-assay CV of 3.7%. Progesterone was measured by radioimmunoassay (Coat-A-Count, DPC, USA), with a sensitivity of 0.02 ng/ml and an intra-assay coefficient of variation of 3.6%.

2.3. Statistical analyses

The distribution of lambing pattern was tested by ordinal regression including as independent variables treatment, parity and their interaction. The effect of treatment on lambing rate was analyzed by a two-way ANOVA including the variables treatment, parity and their interaction. The effect of treatment on birth weight of the lambs from single and twin pregnancies was analyzed by a two-way ANOVA. Differences in IGF-I and progesterone concentrations between treatments were tested by ANOVA for repeated measurements. Analyses were made using the Statistical Analysis System (SAS Inst. Inc., Cary, NC).

3. Results

IGF-I concentrations did not differ between groups before bST treatment. In control ewes, IGF-I concentrations increased around the time of estrus (day 6 to the day of estrus; Fig. 1) and returned to basal concentrations on day 2 of the estrous cycle. In contrast, in bST-treated ewes, IGF-I concentrations increased 2 days after treatment and remained greater ($P < 0.01$; Fig. 1) than those of the control ewes throughout the 6 days post-estrus.

Progesterone concentrations in the estrous cycle following synchronization of estrus did not differ ($P > 0.05$) between groups. All ewes showed estrus and were mated after the estrous synchronization protocol.

Treatment with bST increased ($P < 0.01$) the proportion of ewes lambing twins or triplets (Table 1) with no effect of parity on this variable ($P > 0.10$). Overall, the proportion of ewes lambing was not affected by bST treatment ($P > 0.05$). However, there was an interaction between treatment and parity of the ewes ($P < 0.05$) where bST increased lambing rate in multiparous (bST, 92% compared with control, 67%) but not in first mated ewes (bST, 71% compared with control, 87%).

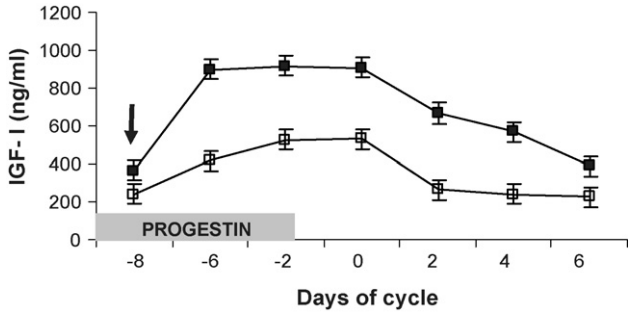

Fig. 1. Plasma concentrations of IGF-I in ewes treated (solid squares) or not (hollow squares) with 125 mg of bST (arrow) 5 days before progestin sponge withdrawal. IGF-I concentrations differ between groups ($P < 0.01$). Day 0 corresponds to the day of estrus. Values are given as least square means ± S.E.M.

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Table 1
Proportion of ewes lambing either singletons, twins or triplets from ewes treated or not with bST (125 mg) 5 days before progestin sponge withdrawal

Groups	*n*	Percent of ewes lambing			Lambs born per ewe lambing
		Singletons	Twins	Triplets	
bST	47	43.5	48.7	7.7	1.64
Control	45	74.3	25.7	0	1.25

The lambing pattern differed between groups ($P < 0.01$).

Birth weight was affected ($P < 0.05$) by the number of lambs born (singleton, 2.98 ± 0.09 compared with twin, 2.39 ± 0.09 kg). Ewes carrying singleton pregnancies treated with bST tended to deliver ($P = 0.08$) heavier lambs (3.16 ± 0.14 kg) than the non-treated ewes (2.81 ± 0.12 kg).

4. Discussion

A single dose of bST 5 days before progestin withdrawal increased the number of lambs born per ewe lambing. The increased prolificacy was associated with increased circulating concentrations of IGF-I in the periovulatory period.

To our knowledge this is the first report where the use of bST increases prolificacy in ewes and constitutes a practical method to improve prolificacy under field conditions. Evidence in the literature indicates bST treatment could be used to improve prolificacy in sheep. Early studies using bST reported an increase in the number of antral follicles, ovulations, embryos and on embryo quality after bST treatment in superovulated cattle (Gong et al., 1996). Similarly, in sheep superovulatory treatment together with bST increased the proportion of transferable embryos (Cognié et al., 1992; Folch et al., 2001). Furthermore, bST treatment to repeat breeder cows at the time of artificial insemination increased conception rate (Morales-Roura et al., 2001). Thus, the evidence indicated that bST could have effects on events previous to or after ovulation in ruminants. In the present study, bST was applied 7 days before the expected time of estrous with a 14-day formulation made for cattle. Therefore, although we did not measure growth hormone concentrations, it is assumed growth hormone was in greater concentrations at the time of development of the ovulatory follicle through the development of the embryo to the blastocyst stage.

Flushing is the most common practice to increase prolificacy in sheep. Flushing increases prolificacy by augmenting ovulation rate (Martin et al., 2004). However, the increased prolificacy after hormonal flushing with bST treatment can be related to the physiological effects of growth hormone and IGF-I on follicle development, oocyte maturation or early embryo development. Thus, it is possible that if this approach is combined with a nutritional flushing treatment further increase in prolificacy could be expected and warrants further investigations.

Lambing rate was greater for nulliparous than for parous ewes when no treatment was applied. This coincides with the greater conception rate in nulliparous than parous ewes after embryo transfer (Bari et al., 2003). Similarly, conception rates are greater in heifers than cows (Morales et al., 2000). In the present study treatment, bST increased the lambing rate in multiparous but not nulliparous ewes. Similar interaction of the effect of bST with age was found for dairy cattle (Starbuck et al., 2006) where bST had no effect on heifers whilst improving fertility in dairy cows. Thus, the effect of bST on lambing rate appears to be greater in multiparous ewes perhaps alleviating deleterious effects of previous pregnancies or parturitions.

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In the present study, IGF-I concentrations increased in response to bST treatment, consistent with previous reports in sheep (Davis et al., 1990) and cattle (Gallo and Block, 1990; Bilby et al., 1999). IGF-I concentration increased 2 days after treatment, thus, it was elevated from 6 days before estrus and could have affected the development of the ovulatory follicle. Although not measured, growth hormone concentrations are assumed to have been elevated due to the bST treatment. Whilst growth hormone does not directly stimulate ovarian follicle growth or steroid production *in vivo* (Scaramuzzi et al., 1999), IGF-I is a potent *in vivo* stimulator of both ovarian follicle development and estradiol secretion. IGF-I stimulates granulosa cell proliferation and differentiation (Campbell et al., 1996; Gutierrez et al., 1997) *in vitro* and stimulates ovarian follicular development and steroidogenesis *in vivo* (Scaramuzzi et al., 1999). Although, bST increased the number of antral follicles growing in the ovary (Gong et al., 1997) it has repeatedly failed to increase the ovulation rate in sheep (Davis et al., 1990; Joyce et al., 1998; Scaramuzzi et al., 1999). Hence, the increased prolificacy observed in this study is unlikely to have occurred by an increase in ovulation rate.

The increase in lambs born per ewe is most likely due to an increase in fertilization rate and embryonic survival than to an increase in the number of oocytes released. In cattle, IGF-I favored nuclear maturation *in vitro* (Lorenzo et al., 1994). In addition, both growth hormone and IGF-I increased cleavage rate after *in vitro* fertilization (Rieger et al., 1998; Moreira et al., 2002a). Thus, somatotropin treatment could be acting by supporting the final stages of follicle development and oocyte maturation enhancing the quality of the oocyte.

In the present study, IGF-I concentrations were not only greater before estrus but remained higher for the first 6 days of the subsequent estrous cycle. Therefore, bST treatment could have affected not only the final stages of follicle development and oocyte maturation but indeed fertilization and the early stages of embryo development. In superovulated ewes, treatment with bST increased the proportion of transferable embryos (Cognié et al., 1992; Folch et al., 2001). When IGF-I or somatotropin were added to the culture medium, there was an increase in the proportion of occytes or embryos becoming blastocyst (Palma et al., 1997; Moreira et al., 2002a). In addition, IGF-I has been shown to protect from insults during early embryo development, such as heat stress and ethanol (Jousan et al., 2004). Thus, IGF-I seems to have a protective role in embryo development. Furthermore, delayed embryo development can cause pregnancy failure if the embryo is unable to produce sufficient interferon τ to prevent luteolysis (Thatcher et al., 2003). In agreement with the former, bST treatment at the time of insemination of superovulated ewes increased the proportion of embryos in advanced stages of development at the time of embryo recovery (Rosas et al., 1995). In addition, IGF-I directly stimulated the secretion of interferon τ by the embryo (Ko et al., 1991). Thus, treatment with bST, and its associated increase in IGF-I, might favor fertility by increasing embryo development and its ability to secrete interferon τ.

Previous studies have suggested that the luteotropic effect of bST may be responsible, at least in part, for the increased in pregnancy rates observed in lactating dairy cows treated with bST (Morales-Roura et al., 2001; Santos et al., 2004). In fact, somatotropin as well as IGF-I improve corpus luteum function. *In vitro*, IGF-I stimulated progesterone production by luteal tissue (Sauerwein et al., 1992). *In vivo*, several studies have reported increased peripheral concentrations of progesterone after treatment with bST (Gallo and Block, 1991; Lucy et al., 1994; Morales-Roura et al., 2001). However, in the present study, progesterone concentrations were not affected by somatotropin so the increased prolificacy and lambing rate were independent of the corpus luteum progesterone secretion.

Birth weight of the lambs was not affected by bST in this study. Costine et al. (2005) reported that ewes treated with bST delivered heavier lambs than the non-treated ewes even though they

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did not differentiate between lambs coming from single or multiple pregnancies. In the present study, the lack of differences in birth weight could be due to the increased proportion of multiple pregnancies with the resultant decreased weight of the lambs at birth. Nonetheless, lambs born from single pregnancies tended to be heavier when the ewes were treated with bST before mating. No abortions were observed in sheep used in the present study. Pregnancy diagnoses were not performed, therefore, early pregnancy losses could have not been detected. Nonetheless, as lambing rate was favorable to the bST-treated ewes a deleterious effect of bST on pregnancy is unlikely.

It is concluded that a single dose of 125 of bST 5 days before progestin withdrawal increases lambing rate and prolificacy in sheep. These effects are associated with an increase in circulating concentrations of IGF-I. The results reported herein constitute a practical tool for the improvement of prolificacy in sheep.

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