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Effect of Bovine Somatotropin Administration on Day 11 after Artificial Insemination in Estrus-Induced Ewes during the Non-Breeding Season

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Abstract. The present study aimed to examine the possibility that bovine somatotropin (BST) treatment after artificial insemination (AI) may improve the fertility by the stimulation of corpus luteum (CL) function in seasonally anestrous ewes. The experiment was conducted at the Tawa Field Station, Shibecha-cho in Hokkaido, Japan, during the non-breeding season (May, 1995). Eighty-nine mature (2 to 3 years old) Suffolk and Suffolk-crossed ewes were treated with controlled internal drug release devices containing 0.3 g progesterone (CIDR-G) for 12 days. Ewes were injected intramuscularly with 500 IU PMSG the day before CIDR removal. Eighty-seven ewes were assigned to an insemination of frozen semen on a fixed-time basis (42 to 50 h) after removal of CIDR. On the 11th day after AI (13 days after CIDR removal), 43 inseminated ewes were received 100 mg of recombinant BST, while the remaining 44 ewes were given vehicle as controls. The BST administration appeared to reduce pregnancy (65.1% vs. 72.7%) and lambing (51.2 vs. 65.9%) rates, prolificacy (145.5% vs. 165.5%) and proportion of ewes rearing more than two lambs (47.6% vs. 60.7%), as compared with controls, although there was no signifiant difference. BST-treated ewes had higher plasma GH concentrations (endogenous GH plus exogenous BST) at Day 19 (8 days after BST treatment) as compared with controls in both pregnant and non-pregnant ewes ($P<0.05$). Also, in the control group, plasma GH concentration of pregnant ewes was higher than that of non-pregnant ewes ($P<0.05$). Plasma progesterone (P) and estradiol-17β (E) cencentrations at Day 19 were lower in BST-treated ewes than that of control ewes ($P<0.05$). In conclusion, an administration with 100 mg of BST on Day 11 after AI during the non-breeding season resulted in the suppression of the ovarian function 1 week thereafter. Consequently, the conception rate and lambing rate did not improve by the present BST treatment in the ewe.

Key words: Bovine somatotropin, Artificial insemination, Progesterone, Conception rate, Ewes.

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To induce estrus during the non-breeding season in the anoestrous ewe, the treatment with progestogen using medroxyprogesterone acetate (MAP) sponges or a controlled internal drug release device (CIDR®) was successfully combined with injections of pregnant mare serum gonadotropin (PMSG), resulted in >90% of estrus induction and 50–80% of lambing rate [1–4]. One of the approaches to improve the fertility may be a trial to promote/support luteal function after mating or artificial insemination (AI). In this context, an application of human chorionic gonadotropin (hCG) or gonadotropin-releasing hormone (GnRH)

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was exmained, but failed to increase the fertility [5].

Besides the classical luteotropic roles of luteinizing hormone (LH), it became gradually evident that growth hormone (GH) also takes part in the control of the corpus luteum (CL). The treatments with bovine GH, somatotropin (BST), resulted in an increase in plasma concentrations of progesterone (P) in lactating dairy caws [6, 7], as well as an increase in the size of the CL in heifers [8]. The data suggest that BST (GH) treatment directly stimulates luteal function. Indeed, GH has shown to stimulate P production by human luteal cells [9], and the release of P from the microdialyzed bovine CL *in vitro* [10]. Furthermore, the expression of GH receptor mRNA was shown in the ovine CL [11]. These data suggest that GH may be a direct regulator of luteal function. Recently, a study using hypophysectomized ewes has shown that treatment with GH was as effective as LH in developing and maintaining normal CL function [12]. We further confirmed that GH was more stimulative than LH in the local release of P within the mid-CL by using a microdialysis system (MDS) implanted in the CL of ewes [13].

Thus, the present study aimed to examine the possibility that BST treatment after AI may improve the fertility by the stimulation of CL function in seasonally anestrous ewes. For this purpose, a field trial with ewes was carried out in which BST was administered to estrus-induced and artificially inseminated ewes during the non-breeding season.

Materials and Methods

Animals and treatment

The present experiment was conducted at the Tawa Field Station, Shibecha-cho in Hokkaido, Japan during the non-breeding season (May, 1995).

Pre-treatment for inducing the estrus

Eighty-nine mature (2 to 3 yeards old) Suffolk and Suffolk-crossed ewes were treated with controlled internal drug release devices containing 0.3 g progesterone (CIDR-G: Eazi breed, Carter Holt Harvey Plastic Products Ltd., New Zealand) for 12 days. Ewes were injected intramuscularly with 500 IU PMSG (Serotopin, Teikoku-zoki Co., Japan) the day before CIDR removal. With this treatment of CIDR and PMSG, seasonally anestrous ewes usually showed estrus 18 to 24 h after CIDR removal [5].

Insemination

Semen for AI was collected from mature two Suffolk and a Texel rams and frozen in pellet form. Freezing and thawing procedures and diluent followed the method as described previously [14, 15]. Out of 89 treated ewes, two ewes lost the CIDRs during the insertion period, and therefore these two ewes were excluded from the present study. The remaining 87 ewes were assigned to an insemination on a fixed-time basis (42 to 50 h) after removal of CIDR, without estrous detection. For each insemination, 0.2 ml of frozen-thawed semen containing at least 50×10^6 spermatozoa was deposited in each uterine horn using the Cassou insemination pipette (Z. M. V., France) with the aid of a laparoscope (Storz, Germany). The detail of intrauterine insemination was described by others [16].

BST treatment

On the 11th day after AI (13 days after CIDR removal), 43 inseminated ewes were received intramuscular injection with 1 ml (100 mg) of recombinant BST (Sometribove, Monsanto Co. St. Louis, MO, U.S.A.) suspension made by diluting with 1.5 ml $NaHCO_3$-NaCl for a vial containing 150 mg rBST. The remaining 44 ewes were given 1 ml of $NaHCO_3$-NaCl as controls.

Hormone determinations

Jugular blood samples (5 ml) were collected into a heparinized vacuum container from all ewes at −1, 11, 19 and 58 days after AI. Plasma was separated immediately after collection by centrifugation at 1630 g for 15 min and stored at −20 C until measurement of P, E_2 and GH concentrations.

Steroid hormones were measured after ether extraction with second antibody enzyme immunoassays [17, 18]. GH concentrations were measured by a radioimmunoassay using NIDDK GH-RIA kit [19]. The assay used NIDDK-anti-oGH-2 as the antibody, NIDDK-oGH-I-4 as a standard and bGH (UCB bioproduct i070-bGH; Belgium) as a tracer.

Conception rate

Pregnancy was first diagnosed by plasma P level

(>1 ng/ml) on Day 19 after AI and again by using an ultrasonic scanning instrument (Supereye, SSD 500; Fujihira Co., Japan) on Day 58 after AI. Pregnancy was confirmed by lambing. Prolificacy (number of new-born lambs/number of lambed ewes) was also compared between BST-treated and control groups.

Statistical analysis

Data on pregnancy (Days 19 and 58) and lambing rates were analyzed by analysis of variance using general linear models (GLM) procedure of Statistical Analysis System (SAS). Prolificacy and mean plasma P, E_2 and GH concentrations were analyzed by ANOVA followed by Student's *t* test.

Results

Conception rate

Lambing rates of ewes inseminated with frozen-thawed semen from 3 different rams were not significantly different with 79.3, 73.3 and 53.6%, respectively. Therefore, the lambing results were pooled to compare the effect of BST treatment. The BST administration appeared to reduce pregnancy (65.1% vs. 72.7%) and lambing (51.2 vs. 65.9%) rates in comparison with the control, although there was no significant difference.

Prolificacy

The BST administration also appeared to reduce prolificacy (145.5% vs. 165.5%) and proportion of ewes rearing more than two lambs (47.6% vs. 60.7%), as compared with controls, but there was no significant difference between treated and control ewes.

Hormonal profile

Plasma GH concentrations at Days 11 and 19 in control and BST-treated ewes were shown in Fig. 1. At Day 11, GH concentrations were not significantly different between pregnant and non-pregnant ewes, and between control and treated ewes. However, BST-treated ewes had significantly ($P<0.05$) higher GH concentrations at Day 19 in both pregnant and non-pregnant ewes as compared with controls. Also, in the control group, plasma GH concentration of pregnant ewes was significantly ($P<0.05$) higher than that of non-pregnant ewes.

Plasma P concentrations in pregnant and non-pregnant ewes are shown in Fig. 2. In both groups, plasma P concentrations at Day 19 were higher in control ewes than those of BST-treated ewes with significant difference ($P<0.05$).

As shown in Fig. 3, plasma E_2 concentrations at Day 11 in BST-treated and control ewes were not significantly different in both pregnant and non-pregnant ewes. However, BST-treated ewes had significantly ($P<0.05$) lower E_2 levels at Day 19 in both pregnant and non-pregnant ewes.

Discussion

In this study, we examined the effect of BST treatment on Day 11 after AI on the conception rate in the estrus-induced ewes during the non-

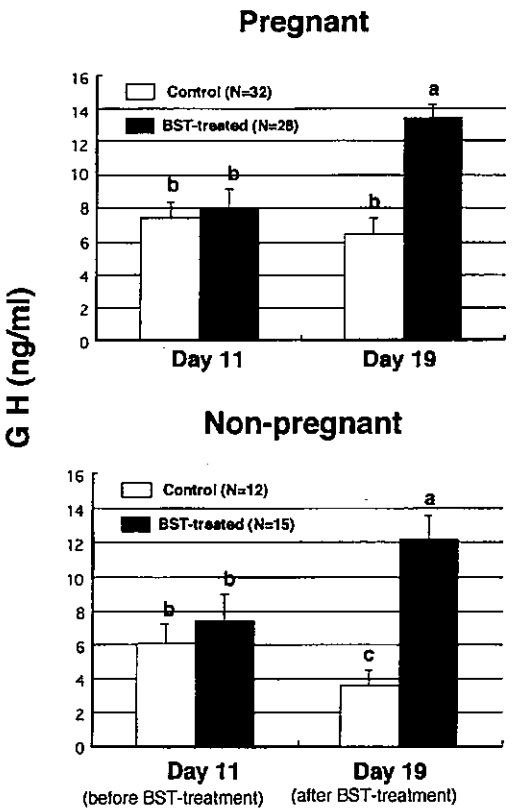

Fig. 1. Effects of intramuscular administration of BST (100 mg) on Day 11 after AI on the plasma GH concentration on Day 19 (8 days after BST-treatment) during the non-breeding season. Values are expressed as mean ± SEM. a, b, c; $P<0.05$.

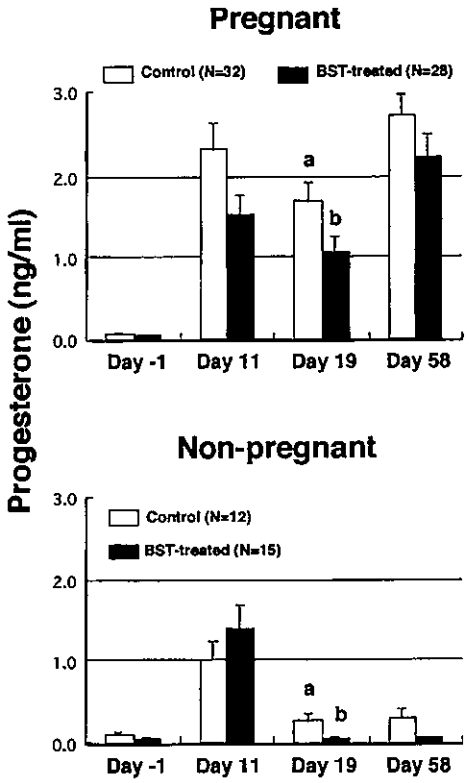

Fig. 2. Effects of intramuscular administration of BST (100 mg) on Day 11 after AI on the plasma P concentration on Days −1, 11, 19 (8 days after BST-treatment), and 58 during the non-breeding season. Values are expressed as mean ± SEM. a, b; P<0.05 on the same day.

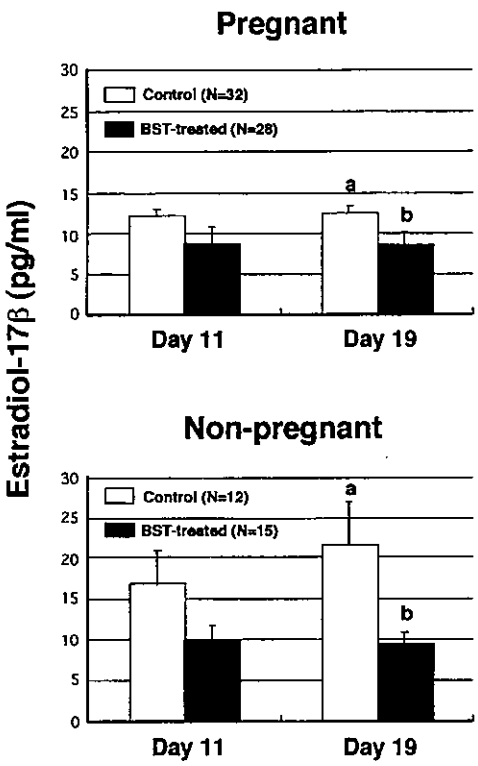

Fig. 3. Effects of intramuscular administration of BST (100 mg) on Day 11 after AI on the plasma E_2 concentration on Day 19 (8 days after BST-treatment) during the non-breeding season. Values are expressed as mean ± SEM. a, b; P<0.05 on the same day.

breeding season. However, the BST treatment with the present time schedule failed to improve the conception rate. Although BST was stimulative in the local P secretion in the *in vivo* MDS implanted in the CL of ewes in our previous report [13], a BST administration to the ewe on Day 11 after AI appeared to suppress the ovarian activity. The facts that plasma levels of both P and E_2 were suppressed 8 days after the treatment with 100 mg of BST suggest that the BST inhibited the ovarian steroidogenesis. The result was opposite to the most of reports that BST stimulates ovarian function if administered with the commercial time schedule (every 2 weeks by 500 mg BST injection intramuscularly with a form of emulsion that releases BST about 36 mg/day) for milking cows. It is thus possible that the present 100 mg dose (3-fold dosage for the cow if compared on the basis of amount of release per day) as an intact form administered 11 days after AI in the ewe was over-dose that might negatively affect the ovarian function. This speculation is partly supported by the lower conception rate and lambing rate in the BST-treated group, though there was no statistical difference between control group.

Alternatively, the timing of the CL stage receiving BST exposure might not be optimal. Namely, the present time schedule was based on the idea that the BST treatment may rescue the less active CL from the regression that occurs normally several days after the treatment if fertilization was not succeeded. It seems that the BST exposure to such CL at late stage may be no longer stimulative enough on the CL function. Therefore, the better time schedule may be based on another idea that the BST treatment may

stimulate the development of newly-formed CL after ovulation. Indeed, an every 4 h-injection with the same BST material at dose of 0.3 mg from Day 5 just after surgery in hypophysectomized ewes resulted in the formation of the CL on Day 12 that was well comparable with control or LH-treated ewes [12]. This result again points out the possibility that 100 mg BST/ewe could be over-dosage. Namely, the above study used only 1.8 mg BST/day/ewe, which was almost 1.8% of the dosage used in the present study.

It is interesting to note that plasma GH concentration in control ewes without BST-treatment was higher at Day 19 after AI in the pregnant group (with a persistent CL) than that of non-pregnant group (with a regressed CL). The data suggest some positive relationship between endogenous plasma GH levels and CL function.

In conclusion, an administration with 100 mg of BST on Day 11 after AI during the non-breeding season resulted in the suppression of the ovarian function 1 week thereafter. Consequently, the conception rate and lambing rate did not improve by the present BST treatment in the ewe. Clearly, the effect of BST dosage must be examined in detailed study. In addition, to investigate the effect of BST treatment on the formation of CL, the trial including the dosage on Days 3–5 during the early CL development after AI should be performed in the future study.

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