



# Effect of growth hormone on milk yield and reproductive performance of subfertile Holstein cows during extended lactations

Jessica Flores<sup>1</sup>, José E. García<sup>1</sup>, Jesús Mellado<sup>1</sup>, Leticia Gaytán<sup>2</sup>, Ángeles de Santiago<sup>2</sup> and Miguel Mellado<sup>2</sup>

<sup>1</sup>Autonomous Agrarian University Antonio Narro, Dept. Veterinary Science, Torreon, 27054 Mexico. <sup>2</sup>Autonomous Agrarian University Antonio Narro, Dept. Animal Nutrition, Saltillo, Coahuila, 25315 Mexico.

## Abstract

The objective of this study was to determine the effects of the administration of recombinant bovine somatotropin (rbST) throughout lactation to high-yielding subfertile (conception > 270 days postpartum) Holstein cows undergoing extended lactations ( $\geq 480$  d) on milk production and reproductive performance. The study used two large adjacent commercial herds with similar management in a hot area of northern Mexico (25° N). Cows in one herd ( $n=2341$ ) received a 500-mg dose of rbST every 14 d until dried off, starting 60 days postpartum. The other herd served as control ( $n=984$ ). Across parity, rbST-treated cows produced 20% greater milk yield than control cows in 305-d lactations. Across parities, milk yield from 305 d postpartum to the end of lactation was 2734 kg higher in rbST-treated cows than control cows. Across parities rbST-treated cows produced 4777 more kg of milk during the entire lactation (mean 605 d) than the control group (mean 572 d). Conception rates (CR) at first service were only 3.2% and 5.9% ( $p<0.05$ ) for control and rbST-treated cows, respectively. Overall CR was 43.5% and 61.6% ( $p<0.05$ ) for control and treated cows, respectively. Control cows required 2.4 more ( $p<0.01$ ) services per conception than rbST-treated cows. It was concluded that in this hot environment, the use of rbST suits dairy producers because it substantially improves both milk yield and reproductive performance in subfertile high-yielding Holstein cows. By markedly increasing lactation length and lactation persistency farm efficiency is greatly improved due to extended herd life.

**Additional keywords:** conception rate; heat stress; lactation persistency; lactation length; services per conception.

**Abbreviations used:** AI (artificial insemination); BCS (body condition score); CR (conception rates); DIM (days in milk); GH (growth hormone); IGF-1 (insulin-like growth factor-1); rbST (recombinant bovine somatotropin); SC (services per conception); THI (temperature-humidity index); TMR (total mixed ration); WP (voluntary waiting period).

**Authors' contributions:** Data acquisition: JF, LG. Study design and drafted the manuscript: MM. Analyzed the results: JEG. Revised the manuscript and reviewed the pertinent literature: JM, AS.

**Citation:** Flores, J.; García, J. E.; Mellado, J.; Gaytán, L.; de Santiago, A.; Mellado, M. (2019). Effect of growth hormone on milk yield and reproductive performance of subfertile Holstein cows during extended lactations. Spanish Journal of Agricultural Research, Volume 17, Issue 1, e0403. <https://doi.org/10.5424/sjar/2019171-13842>

**Received:** 20 Aug 2018. **Accepted:** 28 Feb 2019.

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**Funding:** The authors received no specific funding for this work.

**Competing interests:** All authors declare that there are no actual or potential conflicts of interest between the authors and other people or organizations that could inappropriately bias their work.

**Correspondence** should be addressed to Miguel Mellado: [mmellbosq@yahoo.com](mailto:mmellbosq@yahoo.com)

## Introduction

The steady genetic selection of dairy cows has brought about cows with high milk yields but reduced reproductive performance under high-input modern production systems (Berry *et al.*, 2016). This is particularly true for high producing dairy cows in hot climates which have to dissipate heat produced by metabolic processes, and metabolic heat production increases as the productive capacity of dairy cows increases. Thus, heat stress compromise physiology and production of dairy cows because of the physiological adaptations employed to prevent hyperthermia (Rhoads *et al.*, 2009).

Both thermal stress and high milk yield reduce fertility due to failure of the ovary to appropriately interact with the hypothalamus and pituitary during the periconception period (Lopez *et al.*, 2005) which leads to reduced expression of estrus (Boer *et al.*, 2010) and defective oocytes/embryos (Leroy *et al.*, 2008). Also, high-yielding cows experience a chronically elevated concentration of non-esterified fatty acids and ketone bodies which are closely and consistently related to reproductive disorders (Opsomer, 2015).

Dairy cows going through the transition period shift from positive to negative energy balance which trigger a cascade of postpartum disorders (Vergara *et al.*, 2014) and immune suppression (Hammon *et al.*, 2006)

and leads to a high risk of removal of cows (due to either culling or death) from the herd. In fact, about 75% of diseases in dairy cows usually occur in the first month after calving (LeBlanc *et al.*, 2006) and the majority of veterinary attention is paid to cows from one week before to ten weeks after calving (Zwald *et al.*, 2004). Thus, clearly, calving presents a considerable welfare risk to a dairy cow and the periparturient diseases are an important factor limiting the profitability of intensive dairy farms. Therefore, minimizing health complications, loss of body condition score and suboptimal ovulation become keystones to accomplish high reproductive efficiency in dairy herds. This could be achieved by making the cows calve less frequently and implementing extended lactations (>18 months) which should improve animal welfare and support an improved longevity.

In order for the farmers to effectively manage voluntarily extended calving-to-calving intervals, cows must experience a considerable lactation persistency and remain non-pregnant for more than five months after calving. One way to enhance lactation persistence is the administration of growth hormone (GH) which increases cell proliferation of the mammary gland (Capuco *et al.*, 2003) and increases blood flow to the mammary gland making higher milk precursors to the gland for milk synthesis (Tanwattana *et al.*, 2003). Therefore, the objectives of this study were a) to measure the effects of the administration of recombinant bovine somatotropin (rbST) on milk yield of cows undergoing extended lactation, b) to examine the effect of rbST on the reproductive performance of cows experiencing extended lactation and, c) to assess the effect of rbST on lactation length of cows in a hot environment.

## Material and methods

### Study herds

Milk yield and fertility records for 2015 and 2016 were obtained from two adjacent large dairy herds (>2000 milking cows) in northern Mexico. The herds were representative of intensive dairy herds in a hot environment with good practices for health and reproductive management. Because of the intense heat load for the most part of the year in this area, thermal stress has a severe adverse effects on the reproductive performances of cows, which causes that a lot of cows get pregnant >300 days in milk (DIM), which leads to extended lactations ( $595 \pm 108$  days for cows included in the present study; mean  $\pm$  SD).

These farms were selected because they were located near one another (similar climatic conditions), had fairly similar nutrition, were milked three a day, health and

management programs were alike and used the Afifarm (Kibbutz Afikim, Israel) as management software. In one of the farms, cows were treated with rbST every 14 days from peak of lactation to dry off, whereas cows in the other farm were not treated with this hormone (control group). Primiparous cows in the rbST-treated cows were 606 whereas multiparous cows were 1735. Primiparous and multiparous cows for the control group were 480 and 504 animals, respectively. Cows were selected to meet the following criteria before inclusion to the study: 1) cows not induced hormonally into lactation; 2) lactation not starting with an abortion, 3) four functional quarters; 4) body condition score (BCS) of at least 3.0 at parturition; 5) accumulation of at least 480 DIM at drying off, 6) good general health, 7) without ovarian or uterine disorders after calving, and 8) conception (after multiple services) >270 DIM (subfertile animals). The experimental design was a two-group retrospective field study enrolling cows either treated (herd A) or not (herd B) with rbST. It is acknowledged that when comparing two different herds, confounding variables are difficult to account for. However, commercial herds are the best option for generating adequate animal numbers to accept or reject the null hypothesis that two treatments are equivalent when using analyses of variance.

These dairy farms are located in northern Mexico (25° N) in a zone characterized by high daytime temperatures in spring, summer and fall (around 40 °C) and intense solar radiation associated with low relative humidity (mean THI=80.0; range 69.8 to 85.0) with an average annual precipitation of 230 mm. Mean temperature for the coldest month (January) is 7.8° C.

### Cows facilities and feeding

Cows were kept in open dirt pens with plenty of shade (about 3.5 to 4.5 m<sup>2</sup>/cow) equipped with fans and a sprinkler system in both barns. Cows were provided with shade also in the feed bunk area. Cows were fed the same total mixed ration (TMR) diet thrice daily. Diet was formulated to meet the nutrient requirements for Holstein cows weighing 650 kg and producing 43 kg of 3.5% fat-corrected milk (NRC, 2001). The forage:concentrate ratio was 50:50 and diets consisted of the following primary ingredients on a dry matter basis: corn silage, alfalfa hay, soybean meal, corn grain and a mineral premix. Cows were given sufficient feed 3 times/d to allow ad libitum intakes with about 5 to 10% feed refusal.

### Reproductive management and variables

Cows in both dairies were vaccinated against brucellosis (strain RB51, BRUCEL<sup>®</sup>, PISA laboratories,

Santa Catarina, Mexico; at six month of age and annually thereafter), leptospirosis (LEPTAVOID-H<sup>®</sup>, Merck Sharp & Dohme Corp., Mexico DF), bovine viral diarrhoea, infectious bovine rhinotracheitis, bovine respiratory syncytial virus, and para-influenza (CattleMaster Gold FP5<sup>®</sup>, Zoetis, Mexico DF). The herd veterinarians examined fresh cows to detect and treat cows with postpartum reproductive disorders as well as ketosis (urine ketone evaluation with Ketostix, Bayer Corp. Diagnostics Division, Elkhart, IN, USA).

Direct observation of estrus was used for heat detection aided by tail chalk that was applied daily. After a voluntary waiting period (VWP) of 50 days postpartum, cows observed to be in estrus were artificially inseminated. Cows in estrus during the morning were submitted for artificial insemination (AI) that afternoon and cows in estrus during the afternoon were submitted for AI the next morning. In both herds, controlled breeding programs (Presynch/Ovsynch) were used in all repeat breeding cows to enhance fertility. Commercial frozen-thawed semen from numerous high genetic merit sires from the USA was used across all months of the year. Due to a very low conception rate (CR) experienced in these cows, the majority of cows continued to be inseminated beyond 270 DIM; the range for number of services was 1-23 for the rbST-treated cows and 1-24 for the non-rbST treated cows (services before cows became unprofitable to keep them).

Pregnancy diagnoses were performed at 45±3 days from their last recorded AI. Reproductive performance was monitored at a herd-level based on first service CR, overall CR, services per conception (SC; only pregnant cows). Dates for calving, first service after parturition and subsequent services were recorded. Reproductive variables used to evaluate fertility were CR at first service, CR after five services, CR to all services, and SC. CR at first service was defined as the number of cows pregnant at their first service divided by the number of cows artificially inseminated for the first time after calving. CR to all services was defined as the number of cows that became pregnant up to 480 days of lactation divided by the number of cows inseminated throughout their lactation. SC were defined as total AI to pregnant cows divided by total pregnant cows.

### Milk recording

A total of 3325 lactating primiparous and multiparous (between first and six lactations) Holstein cows were assigned to the study (rbST=2341, non rbST=984 cows). Cows in one of the herds received subcutaneous injections of 500 mg of rbST (Lactotropina<sup>®</sup>, Eli Lilly Co., Mexico) at 14-d

intervals starting on d 60 ± 3 postpartum and ending at drying off. Cows at both dairies were machine milked three a day (00:00, 08:00, and 16:00 h) and yields were recorded electronically at each milking. Cows were managed to cease lactation from 50 to 65 days before expected parturition or when daily milk yield was less than 20 kg. Cows were categorized as primiparous (parity one) and multiparous (parity ≥ two). Outcome variables were 305-d milk yield, daily milk yield up to 305 days postpartum, DIM, milk yield during the whole lactation, average daily milk yield during the entire lactation and 305-d lactation milk yield persistence [305-d milk yield/(peak milk yield × 305) × 100].

### Statistical analyses

Descriptive statistics were obtained with the FREQ procedure of SAS (SAS Inst. Inc., Cary, NC, USA). Continuous data were analyzed for their Gaussian distribution and coefficients of skewness and kurtosis (PROC UNIVARIATE in SAS) to measure the distribution asymmetry and peakedness. All these variables were normal. Conception data were analyzed using the GENMOD procedure of SAS. The model statement for these dependent variables contained only the effect of treatment; BCS was included as covariate. However, covariates were removed from the model if  $p > 0.10$ .

Data on milk yield as well as various reproductive intervals were analyzed according to a completely randomized design using the PROC MIXED procedure of SAS. The general model used was  $Y_{ij} = \mu + A_i + e_{ij}$ , where  $Y_{ij}$  is the dependent variable,  $\mu$  is the overall mean,  $A_i$  is the effect of the  $i$ th treatment and  $e_{ij}$  is the random residual error.

After limiting the number of SC to cows with a confirmed gestation diagnosis, the effect of treatment on the number of SC was evaluated by the bivariate Wilcoxon rank sum test (proc npar1way of SAS) without adjustment for confounders. For all analyses, significance was set at  $p < 0.05$ .

## Results

### Milk yield

Milk production for the different treatment groups is presented in Table 1. Across parities, lactation length was 30 days longer in rbST-treated cows than cows in the control group. Kaplan-Meier survival curves (Fig. 1) showed that a higher proportion of rbST-treated cows presented greater extended lactations up to 1000 days postpartum compared to non-treated cows. There

**Table 1.** Effect of recombinant bovine somatotropin on different milk production variables (means) in primiparous and multiparous subfertile Holstein cows undergoing extended lactations in a hot environment.

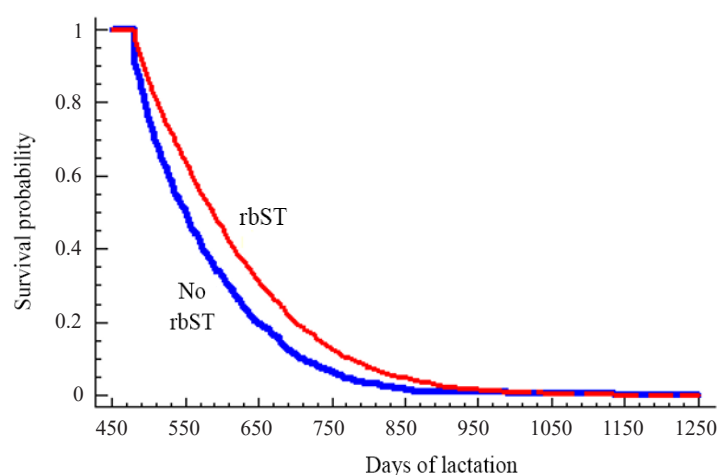
| Milk variables                   | rbST             |                  | No rbST          |                 | SEM  | rbST effect | lact effect | rbST × lact |
|----------------------------------|------------------|------------------|------------------|-----------------|------|-------------|-------------|-------------|
|                                  | 1st lact (n=606) | ≥2 lact (n=1735) | 1st lact (n=480) | ≥2 lact (n=504) |      |             |             |             |
| Lactation length, days           | 604              | 605              | 581              | 563             | 107  | <0.0001     | 0.0391      | 0.0267      |
| 305-d milk yield, kg             | 11676            | 12428            | 9677             | 10926           | 1166 | <0.0001     | <0.0001     | <0.0001     |
| Peak milk yield, kg              | 43.7             | 47.2             | 43.3             | 52.1            | 6.9  | <0.0001     | <0.0001     | <0.0001     |
| Milk yield 305 d to end lact, kg | 9580             | 9531             | 7121             | 6341            | 3735 | <0.0001     | 0.0057      | 0.0146      |
| Total milk yield, kg             | 21256            | 21960            | 16799            | 17268           | 4050 | <0.0001     | 0.0003      | 0.4695      |
| Milk/day in 305 d, kg            | 38.3             | 40.7             | 31.7             | 35.8            | 4.1  | <0.0001     | <0.0001     | <0.0001     |
| Milk/day whole lactation, kg     | 35.2             | 36.3             | 29.0             | 3.8             | 2.7  | <0.0001     | <0.0001     | 0.0009      |
| Milk/day from 305 d to end, kg   | 32.0             | 31.5             | 26.0             | 24.6            | 4.7  | <0.0001     | <0.0001     | 0.0036      |
| Persistence 305-d milk yield, %  | 87.8             | 86.5             | 75.3             | 69.9            | 6.3  | <0.0001     | <0.0001     | <0.0001     |
| Persistence whole lactation, %   | 81.3             | 77.5             | 69.2             | 60.6            | 8.9  | <0.0001     | <0.0001     | <0.0001     |

rbST=recombinant bovine somatotropin; lact=lactation; SEM=standard error of the mean; n= number of observations.

was a parity by treatment interaction ( $p<0.05$ ). The mean lactation length was the same for primiparous and multiparous cows treated with rbST, but lactation length was 18 d longer in primiparous than in pluriparous cows in the control group. Daily milk yield up to 305 d postpartum was significantly different by parity and treatment ( $p<0.01$ ). Across parity, rbST-treated cows produced 2043 kg greater milk yield, which represented 20% more milk than control cows in 305-d lactations. There was a parity by treatment interaction ( $p<0.01$ ) for 305-d milk yield. Difference in milk yield between primiparous and multiparous cows was greater in non-treated cows than in rbST-treated cows. Peak milk yield across parities was 0.6 kg higher ( $p<0.01$ ) in control cows than in rbST-treated cows. There was a parity by

treatment interaction ( $p<0.01$ ). Peak milk yield was 3.5 kg higher in multiparous than primiparous cows in the rbST group whereas this difference was 8.8 kg in the control group.

Across parities, milk yield from 305 d postpartum to the end of lactation was 2734 kg higher in rbST-treated cows than control cows. Nevertheless, the administration of rbST showed an interaction with parity ( $p<0.01$ ) for this trait. While milk yield from 305 d postpartum to the end of lactation was practically similar between primiparous and multiparous cows in the rbST group, primiparous cows produced 778 kg more milk than multiparous cows in the control group. Across parities, rbST-treated cows produced 4777 kg more milk during the entire lactation than the control



**Figure 1.** Kaplan-Meier survival curves for the proportion of cows still milking during lactations  $\geq 480$  days treated with rbST throughout lactation and matched control cows. The survival probability was lower for cows without rbST ( $p<0.001$ ; Wilcoxon test). Median days for ending the lactation was 550 for cows non treated with rbST and 573 for cows treated with rbST.

group. Across parities both 305-d (87% vs 73%) and total lactation (79% vs 66%) persistency were higher for rbST-treated cows compared to control cows. For these traits, there was a parity by treatment interaction ( $p < 0.01$ ). For these traits, multiparous cows showed lower persistency than primiparous cows.

The association between total milk yield for rbST-treated cows and control animals are presented in Fig. 2. A power model was identified as the best-fitting model for the association between total milk yield and lactation length in non-treated cows, whereas a ratkowsky model provided the best fit to these data for the rbST-treated cows, with a clearly greater strength for this later association, implying that 87% of the variation in total milk yield was explained by lactation length in rbST-treated cows.

### Reproductive performance

CR at first service was 2.7 percentage points higher ( $p < 0.01$ ) in rbST-treated cows than control cows (Table 2). Likewise, cumulative CR at fifth service differed ( $p < 0.01$ ) between rbST-treated and control cows. CR to all services was greater ( $p < 0.01$ ) in cows treated with rbST than control cows. Cows not receiving rbST required 2.4 more ( $p < 0.01$ ) SC than rbST-treated cows.

## Discussion

### Milk yield

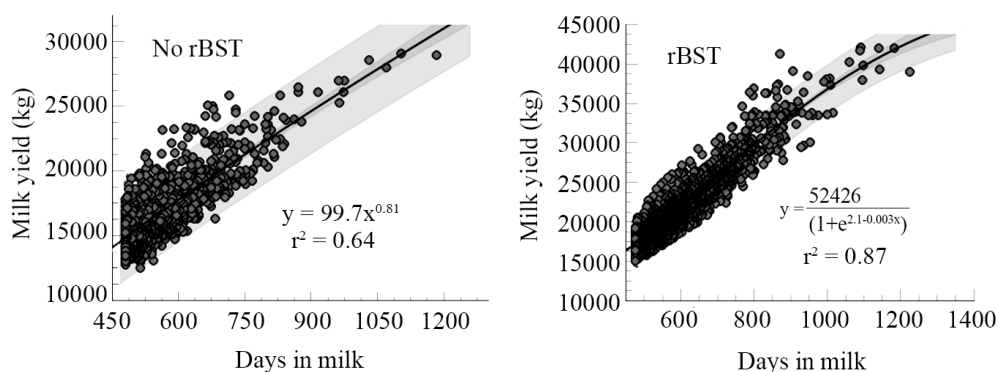
The present study reaffirms the ability of high-yielding Holstein cows incapable of becoming pregnant after several services, due mainly to thermal stress, to present a high lactation persistence which allowed milking of cows for  $\geq 480$ -d when offered adequate energy diets. The extended lactations in the present study were not the result of a conscious decision to

postpone the first insemination after calving, but to the incapability of cows to become pregnant before 200 days postpartum.

Also, this study reiterates that the use of rbST allows each cow to produce an average of 20% additional milk in 305-d lactations which derives from the homeorhetic function of this hormone regulating nutrient partitioning and increasing milk production and improving the efficiency of milk synthesis (Bauman, 1999). rbST induces an increase in blood growth hormone and insulin-like growth factor-1 (IGF-1) concentrations which improves milk yield through increased lipolysis, nutrient prioritization to the mammary gland, and proliferation and maintenance of epithelial cells of the mammary gland (Bauman, 1992). These findings are consistent with previous studies (Burton *et al.*, 1994; Bauman *et al.*, 1999) in which cows receiving rbST have been remarkably consistent in enhancing their milk yield and increases production efficiency.

Nevertheless, the increase in milk yield in the present study in response to 500 mg of rbST administered every other week, was higher than the 8-18% that has been observed for non-heat-stressed cows (Bauman *et al.*, 1999; Santos *et al.*, 2004a; VanBaale *et al.*, 2005). It has also been recognized that the magnitude of the milk yield response to rbST administration is associated to the quality of herd management (Crooker & Otterby, 1991) which demonstrates that farms included in this study had in practice advanced dairy herd management technologies.

In the present study, the effect of rbST was even more pronounced after 305 days postpartum. Cows receiving the hormonal treatment produced 40% more milk from 305 days of lactation to drying off. This was reflected in a 28% increase in total milk yield (median days for ending the lactation in rbST-treated cows was 550). The positive effect of rbST at the end of lactation on milk yield has been also documented by Tarazon-Herrera *et al.* (2000). These results demonstrate that mammary



**Figure 2.** Association between lactation length and total milk yield of high-yielding Holstein cows treated with rbST or not throughout extended lactations in a hot environment.

**Table 2.** Effect of recombinant bovine somatotropin (rbST) on different reproductive variables in subfertile Holstein cows undergoing extended lactations in a hot environment.

| Reproductive variables                  | rbST                          | No rbST                     |
|---|-------------------------------|-----------------------------|
| CR at first service                     | 5.9 (138/2341) <sup>a</sup>   | 3.2 (31/984) <sup>b</sup>   |
| Cumulative CR at fifth service          | 24.7 (578/2341) <sup>a</sup>  | 14.8 (146/984) <sup>b</sup> |
| CR (all services)                       | 61.6 (1441/2341) <sup>a</sup> | 43.5 (428/984) <sup>b</sup> |
| Services per conception (pregnant cows) | 6.6 ± 3.6 <sup>a</sup>        | 9.0 ± 3.7 <sup>b</sup>      |

CR= Conception rate. <sup>a,b</sup>Means in the same raw not shearing the same superscript differ ( $p < 0.01$ ).

cell activity is still increased by exogenous rbST far beyond the 305 d postpartum causing a mild persistence decline well beyond two years of lactation. This prolonged galactopoietic effect of GH in lactating cows derives from the enhancing gene networks that regulate in cell growth and proliferation, transcriptional and translational regulation, actin cytoskeleton signalling, lipid metabolism and cell death (McCoard *et al.*, 2016), and the increase in mammary gland weight through increased cell size (Sciascia *et al.*, 2013). Thus, this strong homeorhetic mechanism of GH to support milk yield during the extended phase of lactation represents an important tool to exploit the productive potential of persistent cows for longer lactation periods. Longer lactating rbST-treated cows on average had not greater peaks than control cows. Lower milk peak yield in early lactation in rbST-treated cows followed by a lactation persistency increase after lactation peak could help to maintain the health of dairy cows during the extended lactation with a marked increase in total milk yield (Yamazaky *et al.*, 2011).

In the present study, rbST treatment resulted in a marked increase in lactation persistence in rbST-treated cows than control cows, a result in line with previous reports in cows treated with rbST (Mellado *et al.*, 2016a). Thus, milk yield was just moderately reduced due to prolonged time spent in late lactation which reaffirms that persistency is plastic, and can be improved by three times a day milking, but more importantly, by the administration of rbST. In the treated group, primiparous cows were more persistent than in pluriparous cows, which is in line with results of Bauman (1999). The marked increased lactation persistency throughout lactations with the administration of rbST offers the opportunity for herd managers to consider extended lactations in areas of intense heat where thermal stress is the main factor affecting reproductive performances in dairy cattle. Also, given that heat stress affects negatively the biological functioning of dairy cows through depressed milk production, the use of

rbST represent a practical and profitable intervention technique to improve milk yield in heat-stressed cows.

## Reproductive performance

In the present study, CR at first service was extremely poor and far lower than the typical target of 45% observed in well-managed Holstein cows in temperate climates (Ferguson & Skidmore, 2013). This low CR at first service is a condition for cows to reach lactation lengths far beyond 18 months. The present study revealed higher CR at first service for rbST-treated cows than control cows. This is explained by the fact that GH improves fertility in cyclic Holstein cows by enhancing developmental competence of bovine oocytes (Kuzmina *et al.*, 2007), reducing embryonic mortality (Santos *et al.*, 2004b) and improvement of uterine health and higher postpartum IGF-1 concentrations (Silva *et al.*, 2017). Also, GH modulates gonadotropin secretion which enhances gonadal function, steroidogenesis and gametogenesis (Hull & Harvey, 2014). It also promotes ovulation and corpus luteum function (Ovesen *et al.*, 1994; Adams & Briegel, 2005).

Heat stress in the present study seemed to be the major factor for a significant reduction in a CR at first service (Mellado *et al.*, 2013) because high ambient temperatures decrease the length and intensity of estrus behaviors, increase the length of anoestrus period and increase the silent ovulation rate (Wolfenson *et al.*, 2000; De Rensis & Scaramuzzi, 2003). These data also suggest that, due to heat stress, a great deal of embryos (Hansen *et al.*, 2001) and fetuses (Mellado *et al.*, 2016b) failed to be sustained as viable pregnancies.

CR after 5 services was still extremely low, with higher percentage of pregnant cows in rbST-treated cows than control cows. These results support the view that supplementation with rbST during the pre- and peri-implantation periods reduced embryonic losses and improve fertility in dairy cows (Ribeiro *et al.*, 2014). These results also suggest a high reproductive wastage post-fertilization (early or late embryonic death) apparently due to intense heat stress during breeding of these cows. Due to the thermal stress experienced by cows for the most part of the year in the present study, extending the VWP seems reasonable in this zone, because, it is expected a very low CR at first service which is a bottleneck in reaching an adequate reproductive efficiency. Also, longer VWP is associated with an increased CR (Tenhagen *et al.*, 2004; Schefers *et al.*, 2010; Lomander *et al.*, 2013).

CR to all services was much higher in rbST-treated cows than control cows. Based on these results, it was evident, that the administration of rbST was connected with higher fertility in high-yielding repeat breeder

cows. This finding is in agreement with other studies (Moriera *et al.*, 2000; Starbuck *et al.*, 2006) that revealed positive effects of a single or repeated rbST injections on reproductive responses in high-yielding cows. It has been suggested that the fertility-promoting effects of rbST may overcome adverse effects of increased hyperthermia on fertility (Jousan *et al.*, 2007). Treatment with rbST also may increase cellular resistance to elevated temperature, either directly or indirectly. Lymphocytes harvested from heifers treated with GH were more resistant to heat shock *in vitro* compared with lymphocytes from nontreated heifers (Elvinger *et al.*, 1991). IGF-1, whose secretion is induced by GH (Bilby *et al.*, 1999), reduced the effects of heat shock on development and apoptosis in preimplantation bovine embryos (Jousan & Hansen, 2004, 2006). Also, Hayhurst *et al.* (2009) reported a significant relationship between circulating concentrations of GH and calving interval. Furthermore, a positive association between serum IGF-1 in the early post-partum period and subsequent cow fertility has been reported (Patton *et al.*, 2007).

Number of SC were lower for the rbST-treated cows than the control cows, although in both groups this variable was far higher than the two or less used as an indicator of good fertility in Holstein herds in southern United States (Washburn *et al.*, 2002). A high number of SC indicates problems with the cow reproductive tract, thermal stress, the work quality of inseminators or the correct detection of estrus. Cows used in this study were high-producing Holstein cows from an area known to have severe heat-stress-related declines in reproduction (Mellado *et al.*, 2013), and with a mean number of SC > 5 (Mellado *et al.*, 2016a); therefore, heat stress for long periods and its residual effects for even longer intervals seems to explain the great difficulty for getting these cows pregnant. The unusually high number of SC required by cows in the present study indicates that it is pointless to inseminate cows during severe heat stress, and would be better to delay the first breeding postpartum until the cow is ready to successfully support a new embryo. This would lead to extended lactations which would benefit the cow, through reduced lifetime exposure to the peak risk period of parturition and early lactation, yet with satisfactory milk yield during the advanced phase of lactation.

In summary, the use of rbST in subfertile high-yielding Holstein cows undergoing lactations lasting 480 to 1200 days and offered TMR diets in a hot environment substantially improved total lactation performance as well as fertility. Therefore, in countries where rbST is legally permitted, the use of this hormone in conjunction with extended lactations is a

promising food production practice which improves agricultural sustainability by improving productivity within the dairy herd and allowing for a reduction in resource use and environmental impact per unit of milk produced. This scheme suits dairy producers in dairies where a surplus of cull cows exists due to prolonged thermal stress because it ameliorates poor reproductive performance and enhances milk yield per year. Additionally, this scheme requires fewer replacement heifers and increase farm efficiency due to additional milk income over feed costs and extended herd life.

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