

Effect of reproductive indices and bovine somatotropin on milk yield in Holstein cows experiencing climatic sub-fertility

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Abstract

This study aimed at determining the association between reproductive indexes and the total milk yield in non-planned lactations ≥ 600 days in sub-fertile Holstein cows treated or not with recombinant bovine somatotropin (rbST) and experienced prolonged hyperthermia. A total of 1141 cows with a single lactation were enrolled in the study: 622 were not treated with rbST (control) and 519 were treated with rbST (500 mg every 14 days throughout lactation). Across parities, control cows with >480 days open, produced 5642 kg more milk ($p < 0.01$) than cows with <380 days open. rbST-treated cows produced 5994 more ($p < 0.01$) kg of milk during the entire lactation than cows with <380 days open. In control cows receiving ≥ 10 services, the average total milk yield was 1811 kg higher ($p < 0.01$) than in cows receiving ≤ 10 services. In rbST-treated cows, the average total milk yield was 1680 kg higher ($p < 0.01$) in cows receiving ≥ 10 services, compared to cows receiving ≤ 10 services. The overall conception rate for all services was 51 % and 73 % ($p < 0.01$) for control and rbST-treated cows, respectively. In conclusion, the greater the delay to get pregnant and the greater the number of services per lactation the higher the total milk yield per lactation were observed. Thus, in this unusual situation where sub-fertile cows are retained with >380 days open and they continue to be inseminated after 10 unsuccessful services, the impact of delayed conception is not entirely negative as this disadvantage is more than outweighed by the extra milk that is produced when lactation is extended.

Key words: pregnancy rate; heat stress; days open; lactation length; services per conception

Introduction

Reproductive performance and milk production in dairy cattle in intensive systems have an important effect on herd economics as faster pregnancies postpartum and higher milk production per lactation increase profits (Cabrera, 2014; Bach et al., 2020). With reduced days open, either with the incorporation of timed artificial insemination or natural estrus, cows produce more milk and are more profitable because most milk is obtained in earlier stages of lactation, close to the peak of lactation which results in greater income over feed cost (Ribeiro et al., 2012; Krpáľková et al., 2020). Also, shorter calving intervals, prolong the productive life of cows and generate more replacements.

However, hyperthermia for most of the year severely disrupts the reproductive efficiency in dairy cows (Hansen, 2019), resulting in a marked decline in pregnancy success per insemination as well as other reproductive metrics. Under these circumstances, attainment of key reproductive efficiency indicators is impossible, which impedes maximization of the number of cows pregnant early in lactation to improve milk production efficiency. Therefore, even with heat abatement mechanisms, herd managers must deal with suboptimal reproductive performance and accept excessive days open in high-yielding cows.

Dairy herds in hot environments have more days open and a considerably low conception rate for all services even with timed artificial insemination programs (Dirandeh et al., 2015; Wolfenson and Roth, 2019), which reduces the overall profits for the dairy producer due to prolonged calving intervals, increased culling rate and higher insemination and replacement costs, as well as loss of genetic gain potential (de Vries and Risco, 2005; Yusuf et al., 2010). Thus, the traditional metrics for reproductive efficiency and lactation length in dairy herds in temperate climates are not always applicable to zones of intense and prolonged ambient temperature, where producers have to adopt more relaxed reproductive indices suitable for zones where the thermoregulatory competence of dairy cows is overwhelmed by severe heat stress and a state of continuous heat gain.

Low conception rates of high-yielding cows due to hyperthermia lead to extended lactations; therefore, lactations beyond 305 days are an inherent feature of cows subjected to air temperature far above their thermo-neutral zone. Results of previous studies measuring the association between milk yield and reproductive performance in non-stressed dairy cows have shown an antagonistic association between these variables, although there is much debate about the certainty of this association (Leblanc, 2010; Mićić et al., 2022). More recent studies have shown that this negative association is small and depends on the level of herd production (Morton et al., 2016; Rearte et al., 2018).

No information is available about the impact of climatic sub-fertility (defined as the reduction in reproductive outcome arising from hyperthermia) in high-yielding Holstein cows on milk production. It was hypothesized that there is no antagonism between poor reproductive performance and milk production in cows not discarded due to failure to conceive and consequently presenting lactations >600 days. Further, it was

hypothesized that the poorer the reproductive performance the greater the total milk yield in cows undergoing extended lactations and receiving recombinant bovine somatotropin (rbST) than in cows not treated with this hormone throughout lactation. Thus, the primary objective of the current study was to assess the associations between various measures of reproductive performance and total milk yield in sub-fertile Holstein cows treated or not with rbST and experiencing lactations ≥ 600 days due to failure to conceive.

Material and methods

Animals and herd management

All procedures involving cows in the present study were approved by the Ethics Committee of the Research Department of the Autonomous Agrarian University Antonio Narro (protocol 42520-3001-2258). This retrospective observational cohort study was carried out on a commercial dairy farm (≈ 4000 milking cows) in a hot-arid environment in northern Mexico (25° N). Weather conditions during the study period ranged from moderate conditions in January (mean maximum temperature 24.7° C) to very hot from March to October (mean maximum temperature 37.1° C). Thus, heat stress for the most part of the year markedly hindered the reproductive efficiency of cows. Effects of heat stress on reproductive performance were immediate during spring and summer but carryover effects influenced reproductive outcomes in the cooler months of late fall, where lingering effects of heat stress are still manifested (Mellado et al., 2013).

Cows with lactations over 600 days due to failure to conceive (sub-fertile) were enrolled in the present study from January 2019 to December 2021. A total of 1141 cows with a single lactation were included in the study: 622 were not treated with rbST and 519 were treated with rbST (500 mg SC, BOOSTIN-S[®] Intervet, CDMX, Mexico, every 14 days from 60 days postpartum to the end of lactation). The herd's annual daily average milk yield production was 34.8 ± 0.27 kg. Cows included in the present study had a body condition score at calving from 3.0 to 3.5 (scale of 1 to 5).

Cows were kept in open dirt pens with ample metal shades in the center of pens and feeding allies. Shade structures in the feeding areas were equipped with stationary fans. Lactating cows were fed ad libitum total mixed rations (TMR) twice per day, and approximately 2.5 % of feed refusals were removed before each feeding. Ration formulation was based on recommendations of the National Research Council (2001) to support milk production of 42 kg/d for cows consuming 25 kg of DM/d. The TMR included corn silage, alfalfa hay, and a concentrate containing corn grain, cotton-seed meal, soybean meal, and minerals. Cows had unrestricted access to fresh drinking water. All cows at the farm were managed under the same conditions and they were dried off 60 days before the expected parturition date or when daily milk production was 20 kg.

Reproductive management

Cows were vaccinated against diseases that provoke pregnancy loss, such as bovine viral diarrhoea, infectious bovine rhinotracheitis, bovine respiratory syncytial virus, and para-influenza (CattleMaster Gold FP5[®], Zoetis, CDMEX., Mexico). Also, cows were vaccinated twice a year against leptospirosis (LEPTAVOID-H[®]; Merck Sharp & Dohme Corp., Mexico, D.F.) Also, all cows were annually vaccinated against brucellosis (strain RB51; BRUCEL[®], PISA laboratories, Santa Catarina, Mexico). The herd veterinarians examined fresh cows after parturition to detect and treat cows with postpartum reproductive disorders, such as retained placenta and uterine infections.

Cows were monitored from 55 DIM for estrus behavior twice daily (visual observation, tail chalk, or pedometer readings) and were artificially inseminated following the AM/PM rule. Eight experienced artificial insemination (AI) technicians performed the inseminations using multiple sires from the USA. No endpoint was established for a cow to express estrus or become pregnant. Mean services per pregnancy in the herd was 4.8 ± 2.8 and cows with three or more inseminations (69.3 % of lactating cows) were subjected to the Ovsynch protocol (100 µg of GnRH; 7 d later 0.5 mg of cloprostenol; 48 h later 100 µg of GnRH and artificial insemination 14-20 h thereafter using frozen-thawed semen). Pregnancy diagnoses were performed at 43 ± 3 days from their last AI. Pregnancy was reconfirmed 45 d later and was followed until calving.

The date of the successful insemination was determined based on the palpation and confirmed from the parturition date. Days open were defined as the number of days from calving to pregnancy. The percentage of pregnant cows with ten services was defined as the proportion of cows that became pregnant and subsequently produced a calf with ten or fewer services. Services per pregnancy were calculated by dividing the total number of services by the number of successful services in pregnant cows.

Milking management and recording

The average lactation number for cows in this study was 2.4 ± 1.7 . Cows were milked thrice daily (0500, 1300, and 2100 hours) and milk yield was recorded electronically at each milking for individual cows. Milk yield was expressed as total milk yield per lactation. Cows completed their tenure when they either reached 210 days of pregnancy or completed their lactation (20 kg/day).

Statistical analyses

Total milk yield in lactations ≥ 600 days was analyzed using a mixed linear model (PROC MIXED of SAS; SAS Institute Inc., Cary, NC, USA) with group (rbST or not) and reproductive metrics as fixed effects, cows as random effect and month of calving, year of parturition and number of lactation as covariates. The interaction group \times class of reproductive performance measures was included in the model. Means

of classes of reproductive performance measures were compared with the PDIF option of SAS. Independent variables were total milk yield per lactation and rbST groups and the interaction between groups (rbST vs. no rbST) and total milk yield.

The effect of the administration of rbST on the association between non-pregnant cows and days open and non-pregnant cows and number of services was analyzed using survival plots generated by Kaplan-Meier survival analysis performed with Statgraphics Centurion version XVII software (Statpoint Technologies Inc., Warrenton, VA, USA). The association between the number of services received per cow and total milk yield was assessed using the CurveExpert Professional 2.5.6 software (Hyams Development, Madison, AL, USA). For all statistical analyses means with $p < 0.05$ were regarded as statistically significant.

Results and discussion

A total of 1141 lactation records from cows experiencing lactations ≥ 600 days were recorded which allowed the establishment of a comprehensive database on a wide range of fertility metrics and milk production (range 15852-45125 kg and 15698-41779 kg, for cows treated or not with rbST). Fertility performance in this study was extremely poor with overall median days open of 482 days, which is very far from the 85-115 days from calving to pregnancy recommended in intensive dairy systems in temperate zones for optimum economic benefits by increasing milk production relative to labor and feed costs (Inchaisri et al., 2010; Cabrera, 2014). The appalling days open in the present study derives from the incapacity of high-yielding cows to become pregnant during 400 days post-calving, mainly due to severe hyperthermia for most of the year. These results show the complication in following general reproductive metrics for dairy operations exposed to extended periods of hot weather.

Table 1 shows that increasing days open increased the total milk yield ($p < 0.01$), with greater ($p < 0.01$) milk production in lactations greater than 600 days in cows receiving rbST. Whilst there is evidence of an antagonistic relationship between the milk yield and reproductive performance (Berry et al., 2003; Madouasse et al., 2010; Piccardi et al., 2013), other reports showed no association, nor a positive neither a negative negligible relationship (Cook and Green, 2016; Rearte et al., 2018; Rethmeier et al., 2019). These inconsistencies seem to result from biased indicators and herd management (LeBlanc, 2010). These previous studies cannot be compared with the present study because they were conducted in zones with adequate climatic conditions for optimum reproductive performance of dairy cows. In the present study, the greater total milk yield with greater days open was not unexpected, as greater days open led to longer lactations and consequently to higher total milk yield per lactation. This is so because of the detrimental effect of gestation on milk production in dairy cattle (Roche, 2003; Leclerc et al., 2008; Penasa et al., 2016) and the need to terminate lactation at about 210 days of pregnancy (van Knegsel et al., 2013). Regardless of

Table 1. The effect of number of days open and the administration of recombinant bovine somatotropin on total milk yield of sub-fertile Holstein cows undergoing heat stress for most of the year

		Growth hormone		p-value
		No	Yes	
Days open	Overall	22276 ± 3861	22672 ± 3764	0.1750
<380	19885 ± 2152 ^a	19104 ± 2012 ^c	20206 ± 2132 ^c	<.0001
380-480	21238 ± 2074 ^b	20295 ± 1735 ^b	21930 ± 2036 ^b	<.0001
>480	25432 ± 3801 ^a	24746 ± 3707 ^a	26200 ± 3771 ^a	<.0001
p-value	<.0001	<.0001	<.0001	

Recombinant bovine somatotropin × days open p=0.66.

the number of days open, cows treated with rbST produced more milk per lactation than non-treated cows, which was expected as rbST improves synthetic capacity of secretory cells and reduces the mammary epithelial cell loss (Annen et al., 2004). The lack of significant interactions between days open and milk production indicated that the positive effect of days open on total milk production in unplanned extended lactations is consistent over treatment with rbST.

For cows not treated with rbST, the total milk yield was 7 % higher in cows that did not become pregnant with 10 services than those pregnant with ≥10 services (Figure 1). Likewise, for cows treated with rbST, total milk yield was 8 % higher in cows unable to become pregnant with 10 services than in cows pregnant with ≥10 services. This response is straightforward because the sooner the sub-fertile cows become pregnant the less extension of lactation. In an ideal situation, the benchmark for services per pregnancy for Holstein herds is around two artificial inseminations (Norman et al., 2009). In the present study extending the number of

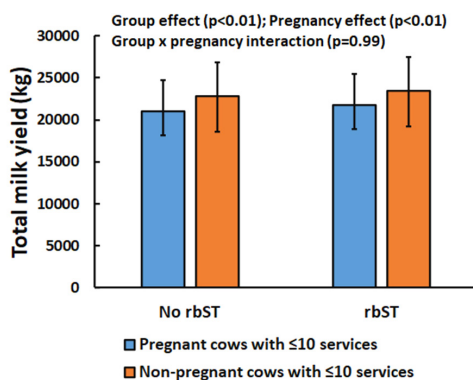


Figure 1. Total milk yield of sub-fertile Holstein cows pregnant with ≤10 services, undergoing heat stress for most of the year, treated or not with recombinant bovine somatotropin, and experiencing lactations ≥600 days

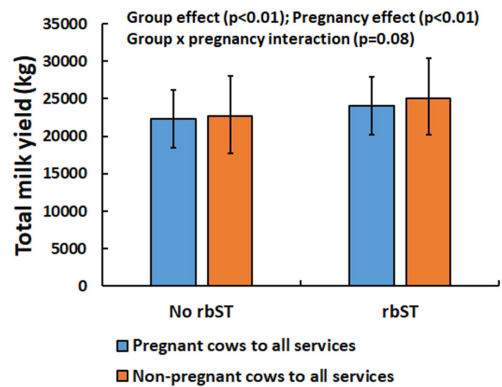


Figure 2. Total milk yield of sub-fertile Holstein cows pregnant to all services (≥10 services), undergoing heat stress for most of the year, treated or not with recombinant bovine somatotropin, and experiencing lactations ≥600 days

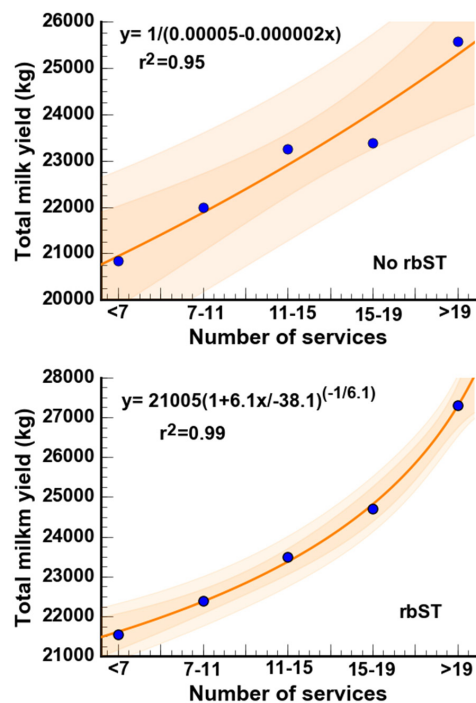


Figure 3. Association between total number of services per lactation and total milk yield of sub-fertile Holstein cows undergoing heat stress for most of the year, treated or not with recombinant bovine somatotropin and experiencing lactations ≥600 days

services beyond 10 markedly increased total milk yield. Even though cows that require various insemination attempts to get pregnant have a higher milk yield per lactation, they are more likely to be culled, reducing their lifetime production, which reduces profitability (González-Recio et al. 2005). These results indicate that healthy cows subjected to prolonged heat stress should not be culled merely because she has

not become pregnant after some “ideal” standard of services. Figure 2 shows that the total milk yield was higher ($p < 0.01$) in cows not pregnant and non-treated with rbST with more than 10 services than in cows pregnant with more than ten inseminations. The same was true for cows treated with rbST, with no services \times group interaction ($p = 0.08$). Mean services per pregnancy was 14.1 ± 4.4 for cows not treated with rbST and 11.1 ± 4.4 for rbST-treated cows. Regardless of pregnancy, total milk yield in unplanned extended lactations markedly increased with the number of services received (Figure 3).

Services administered to cows in the present study were exceptionally high and reflect an unusual situation where prolonged hyperthermia does not allow adequate pregnancy rates. Thus, the question arises, how many times a sub-fertile cow subjected to heat stress should be served before being considered infertile and not poorly managed? Another is how many services should a cow receive before it becomes unprofitable to maintain her in the herd. In the present study pregnancy rates for all services were 51 % and 73 % for cows not treated or treated with rbST, respectively, which are still reasonably acceptable, although after 4 breedings it would be prudent to ascertain the reproductive health of cows. Therefore, an excessive number of services substantially reduced the culling rate of cows and incorporation of replacement heifers even though these sub-fertile cows failed to get pregnant quickly enough which is a common cause of culling cows due to failure to conceive.

On the one hand, cows with 365-day calving intervals are dried off earlier so they produce less milk in the calendar year. On the other hand, failure to conceive in high-yielding cows is more than outweighed by the extra milk that it produces between day 305 and day ≥ 600 . In the present study, the average daily milk yield was 31.4 ± 3.3 and 33.7 ± 3.4 for cows not treated or treated with rbST, indicating a high persistent lactation; thus, the impact of delayed conception did not have a pronounced milk yield loss. Moreover, in practice, the impact of larger numbers of services per conception on the economic loss may differ among herds depending on the size of other expenses associated with reproductive management on dairy farms. These data indicate that the impact of repeated services reflected in delayed conception is not entirely negative in high-yielding cows with highly persistent lactation. Thus, delaying fecundation can have economic advantages (more milk per lactation, less risk of periparturient diseases, and improvement of main reproductive metrics; Niozas et al., 2019a, b; van Kneegsel et al., 2022) even when the calving interval is increased.

Kaplan-Meier time-to-event analysis showed that cows not treated with rbST had a delayed interval from calving to pregnancy compared with cows receiving rbST (Figure 4). In addition, the Kaplan-Meier time-to-event analysis exploring the effect of the number of services on the occurrence of pregnancy of cows was also conducted for each rbST group. The administration of rbST did reduce the number of services needed for the cows to become pregnant compared with cows not receiving rbST (Figure 5). These results demonstrated a clear evidence that the administration of rbST aiming to enhance milk synthesis (Bauman, 1999) and persistence of lactation (Bauman, 1992), also benefited the

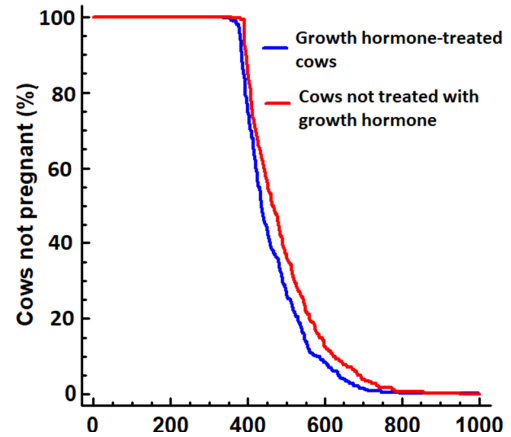


Figure 4. Kaplan-Meier survival analysis of calving-to-pregnancy interval for growth hormone-treated cows or cows not treated with growth hormone and experiencing lactations >600 days due to failure to conceive early in lactation. Cows treated with growth hormone had a median calving-to-conception interval of 435 (95 % CI= 426-447) d compared with 465 (95 % CI= 450-481) d for cows not treated with growth hormone

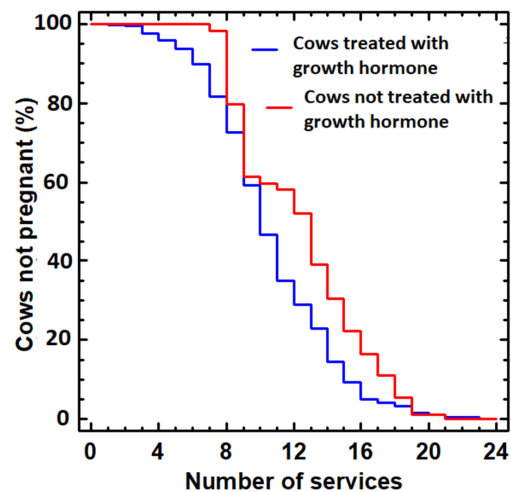


Figure 5. Kaplan-Meier survival analysis of the number of services per pregnancy for growth hormone-treated cows or cows not treated with growth hormone and experiencing lactations >600 days due to failure to conceive early in lactation. Cows treated with growth hormone had a median service per pregnancy of 10.0 (95 % CI= 10-11) d compared with 13.0 (95 % CI= 12-13) d for cows not treated with growth hormone

reproduction performance of sub-fertile cows with extended lactations. These results reaffirmed the marked improvement in reproductive performance with extended lactations and the use of rbST throughout lactation (Flores et al., 2019). Also, these results are in line with other reports where rbST has had a positive impact on fertility of Holstein cows (Santos

et al., 2004; Ribeiro et al., 2014). However, inconsistent with the present results, various reports have demonstrated no improvements in reproductive performance of cows treated with rbST during the prepartum period (Gohary et al., 2014; Silva et al., 2017). These differences arise from the administration of rbST at different periods, physiological stages, and various doses of rbST used.

Conclusions

The results of the present study indicate that the poorer the reproductive performance of high-yielding sub-fertile cows due to prolonged hyperthermia the greater the total milk yield in lactations ≥ 600 days. Thus, involuntary delay of conception in high-yielding cows with very persistent lactation can have economic advantages even when the calving

interval is greatly increased because high-producing cows have a greater chance to remain in the herd and the prolonged calving interval is more than outweighed by the extra milk that cows produce in ≥ 600 days. Moreover, somatotropin treatment throughout the involuntarily extended lactation positively affected the reproductive performance of sub-fertile Holstein cows. Thus, this scheme suits dairy producers in hot climates where rbST is legally permitted because it ameliorates poor reproductive performance and enhances milk yield per year, and extends herd life.

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Učinak reproduktivnih indeksa i primjene govedeg somatotropina na prinos mlijeka u holstein krava koje doživljavaju klimatsku subfertilnost

Sažetak

Cilj ovog istraživanja bio je utvrditi povezanost između reproduktivnih indeksa i ukupnog prinosa mlijeka u neplaniranim laktacijama ≥ 600 dana u neplodnih holstein krava liječenih ili neliječenih rekombinantnim govedim somatotropinom (rbST) i koje su imale produljenu hipertermiju. U studiju je bilo uključeno 1141 krava s jednom laktacijom: 622 nisu liječene rbST-om (kontrola), a 519 je liječeno rbST-om (500 mg svakih 14 dana tijekom laktacije). U svim paritetima, kontrolne krave s >480 dana držanja na otvorenom dale su 5642 kg mlijeka više ($p < 0,01$) nego krave s <380 dana držanja na otvorenom. Krave tretirane rbST-om proizvele su 5994 kg više ($p < 0,01$) mlijeka tijekom cijele laktacije nego krave s <380 dana držanja na otvorenom. U kontrolnih krava koje su primale ≥ 10 tretmana rbST, prosječna ukupna mliječnost bila je 1811 kg veća ($p < 0,01$) nego u krava koje su dobivale ≤ 10 tretmana. U krava liječenih rbST-om, prosječni ukupni prinos mlijeka bio je 1680 kg veći ($p < 0,01$) kod krava koje su primale ≥ 10 tretmana, u usporedbi s kravama koje su primale ≤ 10 tretmana rbST. Ukupna stopa koncepcije za sve tretmane bila je 51 % i 73 % ($p < 0,01$) za kontrolne krave, odnosno 73 % za krave tretirane rbST-om. Zaključno, što je veća odgoda za steonost i što je veći broj tretmana po laktaciji, to je veća ukupna količina mlijeka po laktaciji. Stoga, u ovoj neobičajenoj situaciji u kojoj se neplodne krave drže >380 dana na otvorenom i nastavljaju se osjemenjivati nakon 10 neuspješnih tretmana rbST-om, učinak odgođene koncepcije nije u potpunosti negativan budući da je količina mlijeka proizvedena tijekom produžene višestruko nadmašila eventualne gubitke.

Ključne riječi: stopa steonosti; toplinski stres; otvoreni dani; duljina laktacije; tretmani po začeću

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