FULL ARTICLE

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Productive and reproductive performance of crossbred dairy heifers with induced lactation and efficacy of antimicrobial therapy associated with internal teat sealants*

Desempenho produtivo e reprodutivo de novilhas com lactação induzida e eficácia da terapia antimicrobiana associada com selante interno de tetos

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ABSTRACT

This study evaluated (a) the efficacy of an association between injectable antibiotic therapy and sealant (ATBS) on milk yield (MY), somatic cell count (SCC), and prevalence of intramammary infections (IMI); and (b) the efficacy of gonadotropin-releasing hormone (GnRH) on follicular cyst (FCs) resolution (cyclicity at the 45th day in milk; DIM) and cumulative pregnancy rate (CPR) in heifers submitted to a lactation induction protocol (LIP). A total of 114 crossbred (Holstein × Jersey) heifers, with 34.7 ± 4.8 months and 439 ± 56.35 kg were submitted to LIP. On the 5th day of the LIP, the heifers were assigned to (i) ATBS (n = 57) with 7 mg/kg of norfloxacin associated with sealant and (ii) Control 1 (n = 57; CONT1) with no treatments. Lactation began on the 21st day of LIP and the 15th DIM, FCs were diagnosed and 106 heifers were randomized into two treatment groups with 53 heifers each: (i) GnRH (5 mL injectable GnRH) and (ii) Control 2 (CONT2; no treatment). Of the 114 heifers initially induced, 83.33% (n = 95) responded to LIP with an average MY of 15.19 kg/milk/day during 22 weeks of lactation. In the first 14 DIM, the IMI prevalence was 18% and 28% for heifers ATBS and CONT1 treated, respectively. Additionally, coagulase-negative *Staphylococcus* was the most frequently isolated group of pathogens. Mammary quarters that received ATBS treatment had a lower risk of IMI and SCC than CONT1. The cyclicity at 45 DIM was 68% (ATBS) and 35% (CONT1), and 57% and 46% for animals in the GnRH and CONT2. CPR was 60% in the ATBS group and 89% in CONT1, but GnRH treatment did not affect the CPR. In conclusion, LIP was effective in stimulating MY in heifers, and the IMI prevalence decreased with ATBS treatment. Also, the use of GnRH did not affect the FC regression, cyclicity at 45 DIM, and CPR.

Keywords: Lactation induction protocol. Milk yield. Mastitis. Dairy cattle.

RESUMO

Este estudo avaliou a (i) eficácia da associação entre antibioticoterapia injetável e selante interno de tetos (ATBS) na produção de leite (PL), contagem de células somáticas (CCS), e prevalência de infecções intramamárias (IIM); e (ii) eficácia do hormônio liberador de gonadotrofina (GnRH) na resolução de cistos foliculares (CFs), ciclicidade ao 45º dia em lactação (DEL) e taxa de prenhez cumulativa (TPC) em novilhas submetidas a um protocolo de indução de lactação (PIL). Um total de 114 novilhas mestiças (Holandês × Jersey), com 34,7 ± 4,8 meses e 439 ± 56,35 kg foram submetidas ao PIL. No 5º dia do PIL, as novilhas receberam: (i) ATBS (n = 57) com 7 mg/kg de norfloxacina associada ao selante interno de tetos e (ii) Controle 1 (n = 57; CONT1) sem tratamento. A lactação teve início no 21º dia do PIL e no 15º DEL, foram diagnosticados CFs e 106 novilhas foram agrupadas em dois grupos de tratamento com 53 novilhas em cada: (i) GnRH (5 mL de GnRH injetável) e (ii) Controle 2 (CONT2; sem tratamento). Das 114 novilhas inicialmente induzidas,

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83,33% (n = 95) responderam ao PIL com PL média de 15,19 kg/leite/d durante 22 semanas de lactação. Nos primeiros 14 DEL a prevalência de IIM foi de 18% e 28% para as novilhas tratadas com ATBS e CONT1, respectivamente. Além disso, estafilococos coagulase negativa foram o grupo de patógenos mais frequentemente isolados. Quartos mamários tratados com ATBS tiveram menor risco (0,56) de IIM e menor CCS do que CONT1. A ciclicidade a 45 DEL foi de 68% (ATBS) e 35% (CONT1), e 57% e 46% para os animais no GnRH e CONT2. A TPC foi de 60% no grupo ATBS e 89% no CONT1, porém o tratamento com GnRH não afetou a TPC. Em conclusão, o PIL foi eficaz em estimular a PL em novilhas tardias e a prevalência de IIM diminiuiu com o tratamento ATBS. Além disso, o uso de GnRH não afetou a regressão de CF, ciclicidade em 45 DEL e a TPC.

Palavras-chave: Protocolo de indução de lactação. Produção de leite. Mastite. Gado de leite.

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Introduction

Heifer-raising is an important production cost in dairy farms since in this period there is no income generation (Socha & Johnson, 2000). Considering that onset of puberty in heifers occurs between nine and 11 months of age (approximately 250 and 280 kg for the Holstein breed and between 170 and 190 kg for the Jersey breed), it is expected that the first calving and, consequently, the first lactation occurs around 20-24 months of age (Sejrsen & Purup, 1997; Van Amburgh et al., 1998). Therefore, non-pregnant heifers (>24 months old, and non-lactating) should be avoided in dairy herds because it is not economically desirable, as heifers account for a significant share of the feed and other farm costs. Among the standout factors that can lead to reproductive and productive failures in heifers are management problems, nutrition, and heat stress to diseases (Al-Katanani et al., 1999; Lucy, 2001; Moussavi et al., 2012; Schrick et al., 2001). An alternative to starting milk yield (MY) in unbred heifers is lactation induction (Macrina et al., 2011b).

The lactation induction protocol (LIP) is an association of exogenous hormonal administration that simulates the physiological and hormonal changes that occur between the final third stage of pregnancy and calving. When LIP is used properly, there is an increase in the productive life of adult cows or anticipation of MY in heifers, without milk changes (Lakhani et al., 2017). The majority of the induction protocols use exogenous administrations of estradiol and progesterone, whether or not associated with other exogenous hormones such as prostaglandin, glucocorticoids (dexamethasone), and growth hormone. However, success rates of LIP in heifers range from 60 to 100%, with an average of MY of 11.7 kg/milk/day (Ramgattie et al., 2014).

Despite the potential benefits of LIP, some cows and heifers may have a follicular cyst (FC) due to hormone administrations, which negatively affects reproductive performance (Freitas et al., 2010). In addition, after LIP, there is an increased risk of mastitis, similar to cows with conventional lactations. Thus, treatment with antibiotics and internal teat sealant (ITS) before the first lactation in heifers aims to decrease the prevalence of intramammary infections (IMI) on the early lactation (Naqvi et al., 2018). But there is no scientific data related to the efficacy of injectable antibiotic therapy used in combination with ITS on mammary gland health of heifers submitted to LIP.

Therefore, the present study hypothesizes that LIP is effective in inducing lactation in non-pregnant heifers, and the treatment with injectable antibiotic therapy used in combination with ITS decreases the IMI prevalence and SCC at the beginning of lactation. Additionally, the use of gonadotropin-releasing hormone (GnRH) after LIP as a treatment for FCs reduces their occurrence in heifers. To assess these hypotheses, the objectives were to evaluate (a) the efficacy of an association between injectable antibiotic therapy and sealant (ATBS) on MY and composition; the prevalence of IMI and SCC, and (b) the efficacy of GnRH on FC regression and cumulative pregnancy rate in heifers submitted to LIP.

Material and Methods

Location and animals

The study was conducted on a commercial dairy farm in Itararé, São Paulo, Brazil, from March to September 2016. The climate in this location is tropical and the average temperature is 19ºC with an average annual rainfall of 1306 mm.

A total of 114 heifers were selected among 150 heifers without any previous reproductive management based on the following criteria: (a) not lactating; (b) not pregnant; (c) body condition score (BCS) between 2.5 and 3.75, and (d) no diagnosis of clinical disease (e.g., foot problems, mastitis, reproductive diseases, respiratory diseases). After the selection, 114 crossbred Holstein×Jersey heifers with average 34.7 ± 4.8 months of age, 439 ± 56.35 kg body weight (BW), and BCS of 3.00 ± 0.30 were submitted to LIP, composed of administration of exogenous hormones for 21 days. The number of heifers included in this study was obtained based on a convenience sample (i.e., a non-probabilistic sampling method).

The selected heifers were identified by ear tags and housed in a compost-bedded pack barn. During the LIP, diets were formulated according to the National Research Council (2001) recommendations and were based on two formulations, one for dry heifers with ≥ 350 kg BW and one for the first stages of lactation. Diet and water supply were *ad libitum* and all heifers remained under the same environmental conditions throughout the study.

Information collection before LIP

Information such as identification number, age, weight, BCS, number of functional mammary quarters, and reproductive status was collected before the LIP. To determine BW, the heifers went through a 12-h fast, and the weighing was performed using an electronic scale. The BCS determination was performed according to Edmonson et al. (1989) by a previously trained evaluator. First, visual and tactile observation of the amount of subcutaneous fat at pre-defined points (base of the tail, dorsal regions, and ribs) was performed. Subsequently, the heifers were classified on a 5-point scale, with score 1 = extremely thin, score 2 = moderately thin, score = 3 intermediate, score 4 = moderately fat, and score 5 = extremely fat, which can be subdivided every 0.25 spots.

The reproductive status of the heifers was assessed by transrectal palpation with a portable ultrasound (US Mindray Vet 2200) associated with a 7 MHz transrectal probe. Ovarian and uterine morphology were evaluated to detect the presence of corpus luteum (CL) and FC.

Lactation Induction Protocol (LIP)

If it is not mentioned, all products used in this study were from Ourofino Animal Health®, Brazil. The duration of the LIP used in the present study was 21 days and consisted of intramuscular administration of estradiol benzoate, progesterone prostaglandin (PGF2α), dexamethasone, and recombinant bovine somatropin (rBST, MSD Animal Health®) subcutaneously. The administration of the hormones occurred every 24 h, starting at 10:00 a.m. on the first day (Figure 1).

The protocol of LIP consisted of 1) subcutaneous administration in the ischiorectal fossa of 500 mg/heifer/day of rBST on the first, eighth, 15th, and 21st days of LIP; 2) intramuscular administration of 30 mg/heifer/day of estradiol benzoate from the first to the eighth day and 20 mg/heifer/day of the ninth to the 15th day of LIP; 3) intramuscular

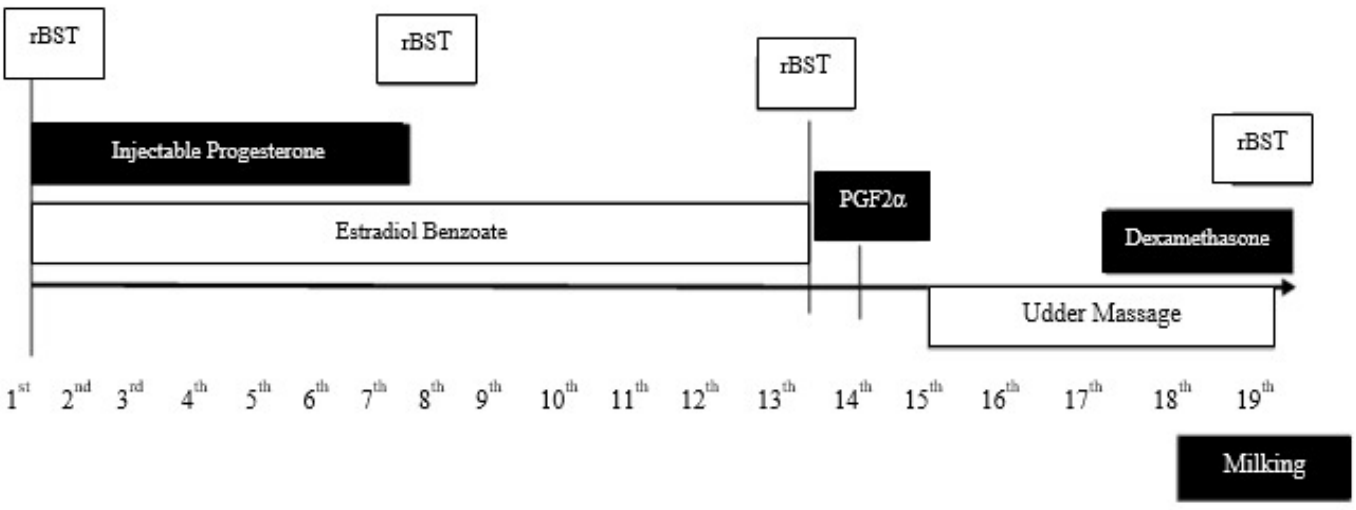

Figure 1 – Lactation induction protocol in heifers. rBST = Recombinant bovine somatotropin; PGF2α = Prostaglandin.

administration of 300 mg/heifer/day of progesterone from the first to the eighth day of LIP; 4) intramuscular administration of 0.530 mg/heifer/day of PGF2α on the 16th day of LIP; 5) intramuscular administration of 20 mg/heifer/day of dexamethasone on days 19, 20, and 21 of LIP; 6) udder massage between the 17th and 20th days of the LIP; 7) start of milking on the 21st day of the LIP, and 8) subcutaneous administration of 500 mg/rBST/heifer every 14 days until the end of lactation (Figure 1).

The udder massages were performed from the 17th to the 20th day of the protocol and aimed to initiate the milking stimulus. From the 21st day after the beginning of the protocol, mechanical milking was performed in all heifers and the lactation was monitored until the 22nd week (150 DIM).

Antimicrobial treatment

On the 5th day of the LIP, the selected heifers were randomly assigned to one of two treatment protocols according to a previously prepared randomized spreadsheet using the RAND function of Excel software (2010, Microsoft Corporation, Redmond, WA, USA). There were 57 heifers in each treatment group: a) ATBS (treated group) with intramuscularly administered 7 mg/kg of norfloxacin used in combination with ITS (4 g of bismuth subnitrate) in each mammary quarter; and b) CONT1 (control group 1) with no treatment. Before the antibiotic administration, the skin of the teat end was cleaned with 70% alcohol to administer the ITS. To avoid injury to the quarter channel, the teat sealant was inserted for approximately 3 mm followed by the administration of a post-dipping solution (0.5% iodine). The first author performed all drug administrations, according to the allocation criteria.

LIP success rate and milk yield

The lactation period started on the 21st day of the LIP. Heifers were mechanically milked twice a day. The milking system was the fishbone type and milking machine from the DeLaval® company. The MY was recorded once a week using an electronic meter (DeLaval®) during 22 weeks of lactation (150 DIM). The LIP success rate was defined as the percentage of heifers that produced ≥ 9 kg/milk/day until the fourth week of lactation according to the studies by Byatt et al. (1997) and Freitas et al. (2010), while the peak of MY was defined as the average of the week in which heifers had the highest MY after initiation of induced lactation.

Milk sample collection and microbiological culture

A total of four heifers were excluded from the study, two heifers due to death and two for hoof problems. Therefore, milk sample collection was completed using 110 heifers. Mammary quarter milk sample collection and microbiological culture analysis were done according to methodologies recommended by the National Mastitis Council (1999).

Milk composition and Somatic Cell Count (SCC)

The milk samples for SCC analysis and milk composition (fat, protein, lactose, total solids, and defatted dry extract) were collected every 15 days, from 15 DIM to 90 DIM, from the milking system using a collecting cup. After collection, the samples were homogenized, identified, and packaged in individual plastic bottles with a 2-bromo-2-nitropropane-1, 3-diol chemical preservative (Bronopol®). Determination of milk fat, protein, lactose, total solids (ST), and milk solids-not-fat (MSNF) content was performed by infrared absorption (Bentley 2000®; Bentley Instruments, Inc., Chaska, MN, USA). The electronic SCC was performed by flow cytometry (Somacount 300® equipment; Bentley Instruments Inc., Chaska, MN, USA).

Diagnosis and treatment of Follicular Cysts (FCs)

Reproductive status was evaluated at 15th DIM in all heifers (n = 110) to detect the frequency of FCs, considered as follicular structures with ovarian diameter ≥30 mm, in the absence of a CL. After diagnosis, 106 heifers were distributed based on BW, age, BCS, and reproductive status (presence/absence of FCs) in two groups: GnRH (n = 53) and CONT2 (n = 53). The GnRH group was treated with intramuscular administration of 5 mL of gonadotropin-releasing hormone (GnRH) in the 15th DIM, while there was no treatment in CONT2. The use of GnRH aimed to promote the involution of FCs and in the untreated heifers, the spontaneous cure of FCs was evaluated at the 45th DIM.

Cyclicity evaluation at 45 days of lactation and Timed Artificial Insemination (TAI)

After 30 days of treatment with GnRH, a second evaluation was performed to evaluate cyclicity, FC regression, and the presence of CL. The volunteer waiting period was 80 days, during which heifers were submitted to a new reproductive status evaluation and TAI protocol.

The TAI steps used were: D0 - Intramuscular administration of 2 mL estradiol benzoate (Ourofino Animal Health®), 2 mL intramuscular PGF2α (Ourofino Animal Health®), 2.5 mL GnRH (Ourofino Animal Health®) and insertion of the intravaginal device with 1g progesterone (Ourofino Animal Health®); D8 - Intramuscular administration of 1 mL estradiol cypionate (Ourofino Animal Health®), 2 mL intramuscular equine chorionic gonadotropin (Ourofino

Animal Health®), 2 mL intramuscular prostaglandin (Ourofino Animal Health®), and removal of the intravaginal progesterone device (Ourofino Animal Health®); D10 - TAI of heifers with conventional semen (Figure 2).

The diagnosis of pregnancy via palpation and transrectal ultrasonography was performed 30 days after TAI. Heifers diagnosed as non-pregnant at 120th DIM were resynchronized at most twice in another TAI. Thus, when necessary, the 2nd TAI was performed at the 120th DIM, and those not pregnant passed the 3rd TAI at the 160th DIM. Intervals between pregnancy diagnostics were approximately 40 days.

After the first pregnancy diagnosis, the first-service conception rate was calculated by the number of heifers that were inseminated and became pregnant. After the three pregnancy diagnoses (160th day of pregnancy), the cumulative pregnancy rate (CPR) was defined as the number of pregnant heifers by the number of heifers inseminated in the 120 days (Figure 3). The effect of GnRH and ATBS treatments on the first conception rate and cumulative pregnancy rate after three TAIs during induced lactation were evaluated.

Data Analysis

The data analyses were performed by Statistical Analysis System® version 9.3 (SAS Institute, Cary, NC, USA). The treatments (GnRH and ATBS administration) were allocated in 2×2 factorial arrangements (2 treatments and 2 controls). The prevalence of IMI, diagnosis of first-service and final pregnancy, and cyclicity were assessed as categorical data by the GLIMMIX procedure, using the heifer as a random effect to consider the correlations of the four mammary quarters within each heifer. For all variables, a statistical significance of $P < 0.05$ was adopted. All response variable averages were calculated and compared by the LSMEANS statement DIFF option using Bonferroni's correction. Assumption of normality of residuals was verified using histograms, studentized residuals graph, and Shapiro-Wilk test. The homogeneity of variances was assessed by Levine's test. Data were examined for outliers and missing values using descriptive statistics and box-plot plots. Non-normal data were transformed (Box-Cox) and outliers of ±3SD (standard deviations) were considered outliers and removed. The SCC data was transformed into SCC linear score (Schukken et al., 2003) to approximate the data to the normal distribution using the Excel program (Microsoft Office, 2016).

Milk yield, milk composition, and FCMY variables were analyzed as repeated measures (for weeks of collection). Several error structures were investigated, and the chosen structure for each variable was evaluated according to Bayesian information criteria (BIC). The following model was considered:

$$Y_{ij} = \mu + Treat_i + Repro_j + Collection_k + BCS_l + Age\ (Covariable) + Weight\ (Covariable) + Treat_i \times Repro_j + Treat_i \times Collection_k + Repro_j \times Collection_k + Treat_i \times Repro_j \times Collection_k + e_{ijkl} \quad (1)$$

Where Y_{ij} = is the observed value; μ = overall mean; $Treat_i$ = fixed effect of antibiotic and ITS treatment i; $Repro_j$ = fixed effect of GnRH treatment j; $Collection_k$ = fixed effect of collection week include k; BCS_l = fixed effect of BCS class 1 (<3.0; between 3.0 and 3.5 and> 3.0); $Treat_i \times Repro_j$ = fixed effect of interaction between treatments for mastitis prevention and FCs; $Treat_i \times Collection_k$ = fixed effect of interaction between mastitis treatments and collection week; $Repro_j \times Collection_k$ = fixed effect of interaction

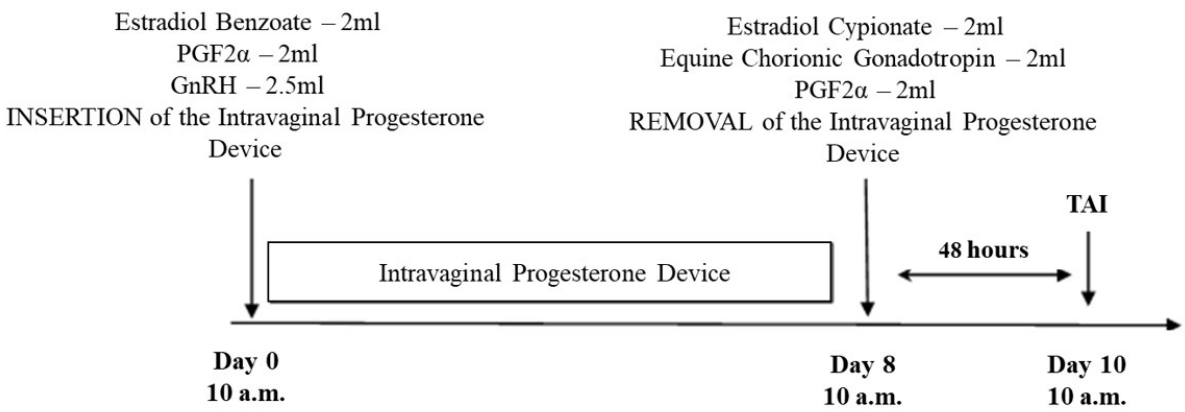

Figure 2 – Timed artificial insemination protocol used in heifers with induced lactation. GnRH = Gonadotropin-releasing hormone; PGF2α = Prostaglandin; TAI = Timed artificial insemination.

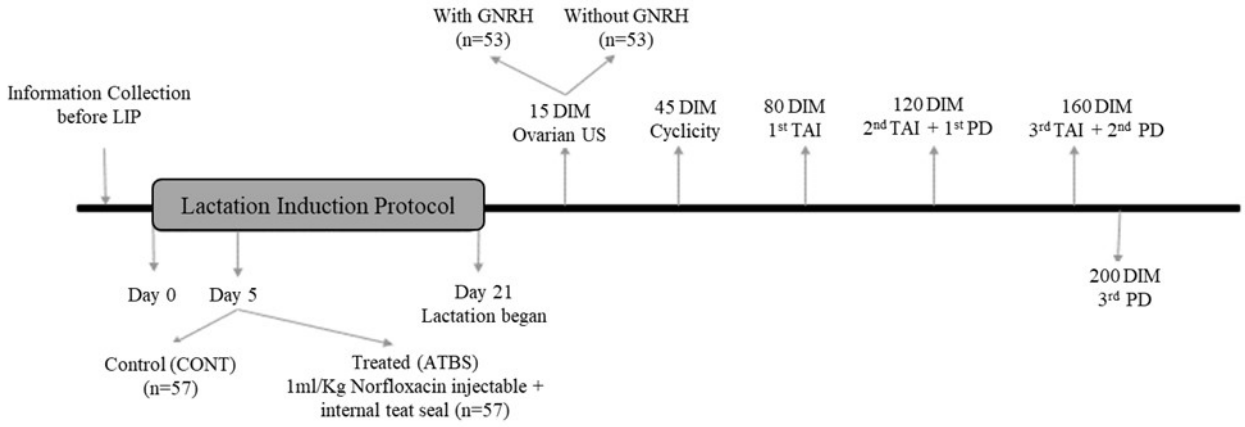

Figure 3 – Trial period flowchart. ATBS: injectable antibiotic therapy associated with internal teat sealant; CONT: Control group without ATBS administration; DIM= day in milk; GnRH: gonadotropin-releasing hormone; LIP= lactation induction protocol; PGF2α = Prostaglandin; PD= diagnosis of pregnancy; US= ultrasonography.

between treatment for FCs and collection week; $Treat_i \times Repro_j \times Collection_k$ = fixed effect of interaction between treatments for mastitis, and FCs and the week of collection and e_{ijk} = random error associated with each observation. The SCC data were transformed into a logarithmic scale to obtain normal distribution and the age and weight variables were included as covariates. The BCS effect did not enter the model when this was the analyzed variable.

The analyses of the prevalence of IMI were performed at the fourth-quarter level, considering the model:

$$\text{logit (pi)} = \beta0 + \beta1 \times \text{Treat} + \beta2 \times \text{Week} + \beta3 \times (\text{Treat} \times \text{Week}) + \beta4 \times \text{Quarter} + \text{Heifer (Random)} + \text{Re} \quad (2)$$

Where logit (pi) refers to the function of the probability of prevalence of IMI (1 or 0); β0 is the intercept; β1 is the regression coefficient for intramammary treatment (Treat); β2 is the regression coefficient of the week of visit (Week); β3 is the regression coefficient for Treat × week interaction, and β4 is the regression coefficient for the quarter position. Heifer was considered as a random effect to take into account the correlation between quarters of each heifer. First-order autoregressive error structure was used and Re is the term residual of the model.

The reproductive performance variables were evaluated considering the fixed effects of ATBS treatment and TAI protocols, and their interactions, according to the model:

$$\text{logit (pi)} = \beta0 + \beta1 \times \text{Treat} + \beta2 \times \text{Prot} + \beta3 \times (\text{Treat} \times \text{Prot}) + \text{Initial Weight (Covariable)} + \text{Initial Age (Covariable)} + \text{Initial BCS (Covariable)} + \text{Cyst 15 DIM (Covariable)} + \text{Cyclicity 15 DIM (Covariable)} + \text{Re} \quad (3)$$

Where logit (pi) is the probability of conception in the first TAI or final diagnosis (1 or 0); β0 = intercept; β1 is the regression coefficient for intramammary treatment (Treat); β2 is the regression coefficient of the reproductive protocol (Prot); β3 is the regression coefficient for Trat × Prot interaction. The variables weight, age, and baseline BCS evaluated one day before the onset of 21-day LIP, and presence of FCs and cyclicity at 15 DIM were included as covariates in the model and Re is the term residual of the model. Binary distribution with the logistic function was used.

The hazard of pregnancy up to 160 DIM was analyzed by the Cox proportional hazard model. The variable time was the interval in days from end treatments to pregnancy. Heifers sold or dead, or those that did not conceive by 160 DIM were censored. For the analysis of time to pregnancy, heifers were considered pregnant if they were diagnosed pregnant. Survival plots were generated by Kaplan-Meier survival analysis performed using GraphPad Prism version 8.00 for Windows (GraphPad Software, San Diego, CA, USA).

Results

LIP success rate, milk yield, and composition

The LIP success rate, calculated according to the number of heifers with MY of ≥9 kg/milk/day in the fourth week of lactation, was 83.33% (95/114). Lactation started 21 days after the onset of LIP and the mean MY during 22 weeks of lactation was 15.19 ± 2.69 kg/milk/day (Table 1). The ATBS treatment did not affect the MY of induced heifers (P = 0.194). However, the GnRH-treated heifers had a lower MY than those from the other experimental groups (P = 0.046; Figure 4).

A progressive increase of MY was observed until reaching the peak of production (16.22 ± 1.04 kg/milk/day), between the 10th and 11th week of lactation. The average

Table 1 – Effect of ATBS and GnRH treatment on SCC, milk yield, and composition of lactation induced heifers

Component	CONT1[a]		ATBS[b]		Average	SEM[e]	*P*-value		
	CONT2[c]	With GnRH[d]	CONT1	With GnRH			ATBS	GnRH	ATBS*GnRH[f]
MY[g]	15.93	15.26	15.69	13.88	15.19	0.85	0.194	0.046	0.786
FCMY[h]	20.36	19.32	19.99	17.42	19.27	1.00	0.006	<0.01	0.058
Fat (%)	4.89	4.66	4.71	4.69	4.74	0.22	0.438	0.168	0.264
Protein (%)	3.57	3.60	3.47	3.57	3.55	0.05	0.012	<0.013	0.121
Lactose (%)	4.50	4.48	4.44	4.49	4.48	0.05	0.261	0.517	0.218
TS[i] (%)	13.98	13.76	13.70	13.77	13.80	0.26	0.223	0.451	0.171
MSNF[j] (%)	9.07	9.08	8.91	9.07	9.03	0.08	0.031	0.033	0.072
SCC[k] Log	5.64	5.67	5.33	5.49	5.49	0.26	0.013	0.898	0.907

[a]CONT1: control group without ATBS administration. [b]ATBS: injectable antibiotic therapy associated with internal teat sealant. [c]CONT2: control group without GnRH use. [d]GnRH: gonadotropin-releasing hormone. [e]SEM: standard error mean. [f]ATBS*GnRH: interaction between treatments. [g]MY: milk yield (kg/milk/day). [h]FCMY: fat corrected milk (kg/milk/day). [i]TS: total solids. [j]MSNF: milk solids-not-fat. [k]SCC: somatic cell count.

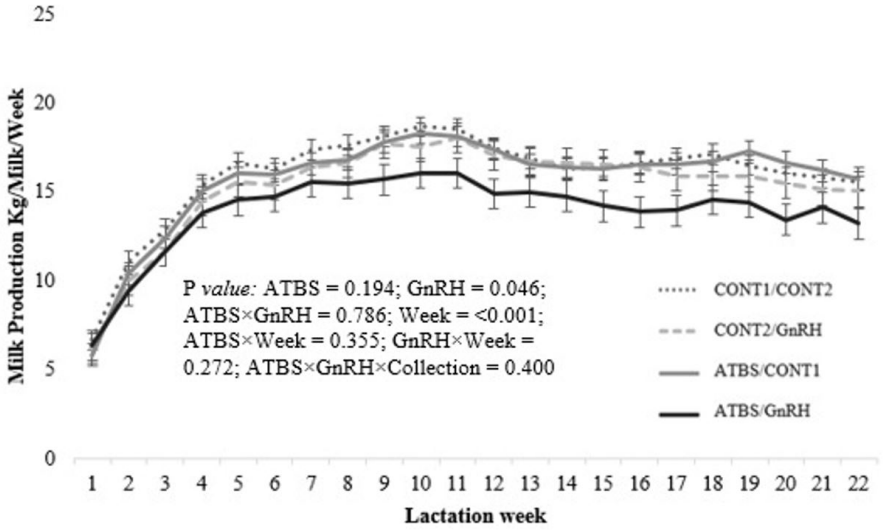

Figure 4 – Effect of ATBS and GnRH on the lactation curve of heifers with induced lactations. ATBS: injectable antibiotic therapy associated with internal teat sealant; CONT1: Control group without ATBS administration; GnRH: gonadotropin-releasing hormone; CONT2: control group without GnRH use.

FCMY (3.5%) biweekly collections during the 90th DIM was 19.27 kg/milk/day (Table 1). Control heifers (CONT1 and CONT2) presented higher FCMY compared to treated heifers (ATBS and GnRH; $P < 0.01$). On the other hand, there was no ATBS × GnRH interaction for MY and FCMY ($P > 0.05$; Table 1).

The average fat, protein, lactose, total solids (TS), and MSNF contents between the CONT1 and ATBS groups were: 4.74%, 3.55%, 4.48%, 13.80%, and 9.07%, respectively, during biweekly milk collection up to the 90th DIM in heifers with induced lactations. There was no effect of ATBS, GnRH and interaction (ATBS × GnRH) treatments on fat ($P \geq 0.168$), lactose ($P \geq 0.218$), and TS ($P \geq 0.171$) contents. Also, there was no effect of interaction (ATBS × GnRH) on protein ($P = 0.121$), and MSNF ($P = 0.072$) contents. On the other hand, effects of ATBS and GnRH treatments were observed on protein content ($P = 0.012$; $P <0.001$, respectively; Table 1). The average milk protein content according to the experimental groups were: 3.57% (CONT1 + CONT2), 3.6% (CONT1 + GnRH), 3.47% (ATBS + CONT2), and 3.57%. (ATBS + GnRH), with higher protein content in the CONT1 + CONT2 and ATBS + GnRH treatments and lower protein content in the ATBS + CONT2 group.

The ATBS ($P = 0.031$) and GnRH ($P = 0.033$) treatments had an effect on the MSNF. The MSNF averages between the experimental groups were: 9.07% (CONT1 + CONT2), 9.08% (CONT1 + GnRH), 8.91 (ATBS + CONT2), and 9.07 (ATBS + GnRH), with lower MSNF contents in the group ATBS + CONT2 (Table 1).

SCC, frequency of mastitis-causing pathogens, and prevalence of IMI

The heifers treated with ATBS had a lower linear score of SCC ($P = 0.013$; Table 1) than the untreated heifers during the first 90 DIM. GnRH treatment did not influence the

linear score of SCC ($P = 0.898$) and there was no effect of interaction ($P = 0.907$).

Of the total mammary quarters sampled in the first two weeks of lactation, 69.16% (305/441) had a negative culture in the 7th DIM and 74.88% (331/442) in the 14th DIM (Table 2). The most frequently isolated pathogens in both milk samples periods were coagulase-negative *Staphylococcus* (CNS) and *Staphylococcus aureus*

Heifers submitted to ATBS treatment had a lower prevalence (18%) of IMI in the first 14 DIM ($P = 0.002$; Table 3) compared to untreated heifers (28%; CONT1). Also, the treated group (ATBS) had a 0.56 times lower risk to develop IMIs than the CONT1 group (untreated) in the first two weeks of lactation.

Return to cyclicity, first-service conception rate, and cumulative pregnancy rate

The reproductive system evaluation was performed in 106 heifers at the 15th DIM and 33.01% (35/106) presented FCs. At the 45th DIM, 68% of the ATBS group heifers were cycling (due to the CL) compared to 35% of CONT1 ($P = 0.004$; Table 4). However, GnRH administration at 15 DIM did

Table 2 – Frequency of isolation of mastitis-causing pathogens on the first 14 days of lactation in lactation-induced heifers

Pathogens	d 7						d 14					
	CONT1[a]		ATBS[b]		TOTAL		CONT1		ATBS		TOTAL	
	n	%	n	%	n	%	n	%	n	%	n	%
Total Samples[c]	212	100.00	229	100.00	441	100.00	215	100.00	227	100.00	442	100.00
Negative	139	65.57	166	72.49	305	69.16	144	66.98	187	82.38	331	74.88
Positive	73	34.40	63	27.51	136	30.84	71	33.02	40	17.62	111	25.12
Contaminated[d]	8	3.77	8	3.49	16	3.52	8	3.72	5	2.20	13	2.92
SCN[e]	54	25.47	41	17.90	95	20.95	52	24.19	24	10.57	76	17.11
S. aureus	6	2.83	5	2.18	11	2.60	6	2.79	5	2.20	11	2.47
Corynebacterium spp.	1	0.47	4	1.75	5	1.10	2	0.93	-	-	2	0.45
Others[f] *Enterobacteria*	-	-	-	-	-	-	-	-	4	1.76	4	0.90
Strep. dysgalactiae	2	0.94	1	0.44	3	0.66	1	0.47	1	0.44	2	0.45
Strep. uberis	-	-	-	-	-	-	2	0.93	-	-	2	0.45
Streptococcus spp.	1	0.47	1	0.44	2	0.44	-	-	-	-	-	-
Pseudomonas spp.	-	-	1	0.44	1	0.22	-	-	-	-	-	-
Strep. agalactiae	-	-	1	0.44	1	0.22	-	-	1	0.44	1	0.22
Non functional mammary quarters	1	0.47	1	0.44	2	0.44	-	-	-	-	-	-

[a]CONT1: Control group without ATBS administration. [b]ATBS, Injectable antibiotic therapy associated with internal teat sealant. [c]Samples per fourth mammary quarters. [d]Contaminated: ≥ two pathogens identified in the same milk sample. [e]SCN: coagulase-negative *Staphylococcus.* [f]Others *Enterobacteria: Enterobacter* spp., *Enterococcus* spp, *Serratia* spp.

Table 3 – Treatment effect logistic regression on the risk of intramammary infection in the first two weeks of lactation of the treated mammary quarters of induced lactating heifers

Risk of IMI[a]	β[β]	SE[c]	*P*-value*	OR[d]	95% CL[e]		LSM[f]	SEM[g]
					Lower	Upper		
Intercept	-2.01	0.269	<0.001	-	-2.541	-1.472	-	-
TREAT[h]								
CONT1[i]			Reference				0.289	0.033
ATBS[j]	-0.881	0.289	0.002	0.565	0.363	0.879	0.187	0.026
Week								
1	Reference						0,263	0,027
2	-0.003	0.249	0.064	0.736	0.530	1.022	0.208	0.025
Mammary Quarter								
RL[k]			Reference				0.196	0.030
RR[l]	0.329	0.237	0.165	1.390	0.873	2.215	0.035	0.191
FL[m]	0.544	0.233	0.019	1.723	1.090	2.724	0.295	0.037
FR[n]	0.046	0.244	0.849	1.047	0.649	1.691	0.203	0.031

[a]IMI: Intramammary infections. [b]β: regression coefficient. [c]SE: standard error. [d]OR: odds ratio. [e]CL: confidence limit. [f]LSM: least square mean. [g]SEM: standard error mean. [h]TREAT: treatment. [i]CONT1: control group without ATBS administration. [j]ATBS: injectable antibiotic therapy associated with internal teat sealant. [k]RL: rear left quarter. [l]RR rear right quarter. [m]FL: front left quarter. [n]FR: front right quarter. **P*-value: ATBS = 0.011; Collection = 0.067; ATBS × Collection = 0.064; Mammary quarter = 0.065.

not affect cyclicity at the 45th DIM ($P = 0.36$; GnRH = 57%; CONT2 = 46%). In addition, there was no effect of the variables weight, age, initial BCS, cyclicity, and FCs at the 15th DIM on the return of cyclicity ($P \geq 0.187$; Table 4).

There was no effect of treatments on the first-service conception rate (ATBS; $P = 0.114$ and GnRH; $P = 0.152$; Table 5). Mean conception rates among experimental groups were 48% for ATBS, 67% for CONT1, 49% for GnRH and 66% for CONT2. The variables weight, age, initial BCS, cyclicity, and presence of FCs in both 15th DIM did not influence the first-service conception rate at 120 DIM ($P \geq 0.165$; Table 5).

The average cumulative pregnancy rate was 75.35%. ATBS-treated animals had a lower cumulative pregnancy rate than CONT1 (60% *versus* 89%; $P = 0.018$; Table 6). GnRH treatment did not change the cumulative pregnancy rate ($P = 0.0509$), and the average for the group treated with GnRH was 64% and 87% for CONT2. The variables weight, age, and BCS in classes at the first stages of lactation did not affect the cumulative pregnancy rate, as well as cyclicity, and presence of FCs at the 15th DIM ($P \geq 0.240$; Table 6).

According to survival analyses, homogeneous curves were observed between treatment groups for hazard of pregnancy up to 160 DIM (ATBS x CONT1: Log-rank = 0.099, Wilcoxon = 0.154, Hazard ratio = 0.847; GnRH x CONT2: Log-rank = 0.165, Wilcoxon = 0.261, Hazard ratio = 0.872). Kaplan-Meier survival plots are shown in Figure 5.

Table 4 – Logistic regression of the effect of ATBS and GnRH treatment, initial weight, age, BCS, 15-day lactation cyclicity, and incidence of follicular cysts on 45-day lactation cycle in heifers with induced lactation

Cyclicity 45	β[A]	SE[b]	*P*-value*	OR[c]	95% CL[d]		LSM[e]	SEM[f]
					Lower	Upper		
Intercept	-0.689	1.990	0.730					
ATBS[g]	1.241	0.647	0.004	3.88	1.54	9.76	0.68	0.08
CONT1[h]	Reference						0.35	0.09
GnRH[i]	0.299	0.625	0.360	1.51	0.61	3.69	0.57	0.09
CONT2[j]	Reference						0.46	0.09
Initial weight[k]	-0.005	0.005	0.329	0.99	0.98	1.00		
Initial age[l]	0.056	0.060	0.349	1.05	0.93	1.19		
BCS[m] 3 e 3.5	0.476	0.538	0.561	1.61	0.55	4.69		
BCS >3,5	0.868	0.913		2.38	0.38	14.64		
BCS <3	0							
FC d15[n]	-0.063	0.475	0.894	0.93	0.36	2.41		
Cyclicity d15[o]	-1.363	1.026	0.187	0.25	0.033	1.96		

[a]β: regression coefficient. [b]SE: standard error. [c]OR: odds ratio. [d]CL: confidence limit. [e]LSM: least square mean. [f]SEM: standard error mean. [g]ATBS: injectable antibiotic therapy associated with internal teat sealant. [h]CONT1: control group without ATBS administration. [i]GnRH: gonadotropin-releasing hormone. [j]CONT2: control group without GnRH administration; [k]Weight before LIP start. [l]Age before the onset of LIP. [m]BCS: body condition score. [n]Presence of follicular cysts on the 15th day of lactation. [o]Cyclicity on the 15th day of lactation. **P*-value = ATBS × GnRH = 0.802.

Table 5 – Logistic regression for the effect of ATBS and GnRH treatment on conception rate after first initial fixed-time artificial insemination in induced lactating heifers

First-service conception rate	β[A]	SE[b]	*P*-value*	OR[c]	95% CL[d]		LSM[e]	SEM[f]
					Lower	Upper		
Intercept	6.340	2.273	0.006					
ATBS[g]	-1.157	0.708	0.114	0.45	0.17	1.21	0.48	0.09
CONT1[h]	Reference						0.67	0.09
GnRH[i]	-1.083	0.715	0.152	0.49	0.18	1.30	0.49	0.09
CONT2[j]	Reference						0.66	0.09
Initial weight[k]	-0.006	0.005	0.276	0.99	0.98	1.00		
Initial age[l]	-0.085	0.061	0.165	0.91	0.81	1.03		
BCS[m] 3 e 3,5	0.029	0.554	0.451	1.03	0.34	3.11		
BCS >3,5	1.036	0.899		2.82	0.47	16.92		
BCS <3	0							
FC d15[n]	0.531	0.518	0.309	1.70	0.60	4.77		
Cyclicity d15[o]	1.189	1.035	0.254	3.28	0.41	25.84		

[a]β: regression coefficient. [b]SE: standard error. [c]OR: odds ratio. [d]CL: confidence limit. [e]LSM: least square mean. [f]SEM: standard error mean. [g]ATBS: injectable antibiotic therapy associated with internal teat sealant. [h]CONT1: control group without ATBS administration. [i]GnRH: gonadotropin-releasing hormone. [j]CONT2: control group without GnRH administration; [k]Weight before LIP start. [l]Age before the onset of LIP. [m]BCS: body condition score. [n]Presence of follicular cysts on the 15th day of lactation. [o]Cyclicity on the 15th day of lactation. **P* value = ATBS × GnRH = 0.457.

Table 6 – Logistic regression for the effect of injectable antibiotic therapy and sealant and GnRH treatment on the cumulative pregnancy rate in induced lactating heifers

Pregnancy rate	β[A]	SE[b]	*P*-value*	OR[c]	95% CL[d]		LSM[e]	SEM[f]
					Lower	Upper		
Intercept	-2.306	7.13	0.747					
ATBS[g]	-1.576	1.16	0.018	0.19	0.04	0.75	0.60	0.11
CONT1[h]	Reference						0.89	0.07
GnRH[i]	-1.274	1.18	0.0509	0.25	0.06	1.00	0.64	0.12
CONT2[j]	Reference						0.87	0.07
BCS[k] 3 e 3.5	-1.221	1.14	0.553	0.29	0.03	2.85		
BCS >3,5	-2.164	2.72		2.52	0.48	16.73		
BCS <3	0							
Initial weight[l]	-0.069	0.05	0.240	0.93	0.83	1.04		
Initial age[m]	2.723	2.48	0.275	15.22	0.10	0.99		
Cyst d15[n]	0.537	0.61	0.387	1.71	0.50	5.85		
Ciclicity d15[o]	0.402	1.18	0.735	1.49	0.14	15.86		

[a]β: regression coefficient. [b]SE: standard error. [c]OR: odds ratio. [d]CL: confidence limit. [e]LSM: least square mean. [f]SEM: standard error mean. [g]ATBS: injectable antibiotic therapy associated with internal teat sealant. [h]CONT1: control group without ATBS administration. [i]GnRH: gonadotropin-releasing hormone. [j]CONT2: control group without GnRH administration; [k]BCS: body condition score. [l]Weight before LIP starts. [m]Age before the onset of LIP. [n]Presence of follicular cysts on the 15th day of lactation. [o]Cyclicity on the 15th day of lactation. *P*-value: ATBS × GnRH = 0.907.

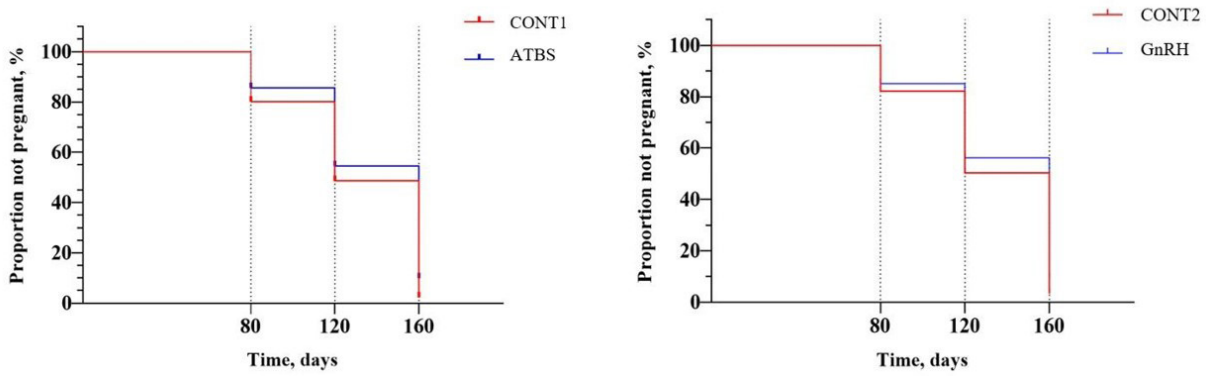

Figure 5 – Kaplan-Meier survival curves for time to pregnancy up to 160 DIM according to treatment groups (CONT1: Control group without ATBS administration vs. ATBS: injectable antibiotic therapy associated with internal teat sealant; and CONT2: control group without GnRH use vs. GnRH: gonadotropin-releasing hormone) of heifers with induced lactations.

Discussion

LIP success rate

LIP success was observed in 83.33% of heifers, suggesting that the LIP used in heifers was efficient in inducing lactation. According to Freitas et al. (2010), even success rates lower than 100% can be profitable due to the low cost to induce the lactation cycle in unproductive cows. The success rate observed in the present study was higher than previous success rates reported in other studies of cows and induced heifers: 70% with 13 to 16 month-old Holstein heifers (Byatt et al., 1997), and 62% with Holstein heifers 24.5 months old (Smith & Schanbacher, 1974). However, the success rate in the present study was lower than that described in Holstein and Jersey heifers aged 22 to 32 months (100%; Fulkerson, 1978), Holstein heifers aged 15 (97%; Macrina et al., 2011b), and 30 months (92%; Harness et al., 1978) and also in Holstein cows (100%; Mellado et al., 2006). But the high success rates described in other studies may be associated with less strict criteria (Ramgattie et al., 2014), such as MY ≥ 5.6 kg/milk/day at lactation peak, which may lead to successful management 100% (Fulkerson, 1978), and which differed from the MY threshold used in the present study (≥ 9kg/milk/day in the first four weeks of lactation).

Milk yield

To our knowledge, no previous studies used hormone protocols similar to the present study in unbred heifers. However, our results are similar to the levels of MY observed in heifers aged 18 months submitted to LIP (11 to 15 kg/milk/day for 22 weeks; Ramgattie et al., 2014). Other studies

with LIPs in crossbred heifers described lower mean MY responses than our study at 6 kg/milk/day (Fulkerson & McDowell, 1975) and 7 kg/milk/day (Fulkerson, 1978). Another study with Holstein dairy heifers aged 18 months resulted in a higher mean MY than our study, which was 18.9 kg/milk/day at 305 DIM (Macrina et al., 2014). Nevertheless, variations in the success rates of LIP and MY in heifers may occur due to incomplete simulation of physiological changes occurring between late third and calving stages in cows and heifers (Ramgattie et al., 2014). Other variables may also affect the MY and LIP success rate, such as the estrous cycle phase at the beginning of the LIP, the pharmacological basis applied and the doses of hormones used (Freitas et al., 2010; Smith & Schanbacher, 1973).

The ATBS did not affect MY of LIP-treated heifers. Studies using antibiotic and/or sealant treatment before the first lactation in primiparous cows with conventional lactations also reported no effect on milk yields using different antimicrobials: lactating cow intramammary antibiotics (pirlimycin hydrochloride, Middleton et al., 2005; and cephapirin, Borm et al., 2006) injectable antibiotic (penethamate hydriodide; Passchyn et al., 2013) and intramammary antibiotic formulated for lactating cows associated with ITS (Machado & Bicalho, 2018). On the other hand, an increase in MY was observed in primiparous cows treated before the first lactation with intramammary antibiotics with formulations for dry cows (Sampimon et al., 2009; Trinidad et al., 1990) and injectable antibiotics for lactating cows (Kreiger et al., 2007; Oliver et al., 2003). The present study hypothesized that ATBS-treated heifers would have increased the MY compared to control heifers (CONT1). However, one of the potential causes for the non-increase in MY of heifers may be due to the distinct types of genetics, management, medications used, prevalence, and type of pathogens present in the mammary glands of animals used in the present study (Naqvi et al., 2018; Passchyn et al., 2013).

Unexpectedly, GnRH treatment, which aimed to cure FCs, negatively influenced MY. No studies are reporting that MY decreases in cows with induced lactation after the use of GnRH. One explanation may be due to the metabolic overload that heifers submitted to induced lactation protocols and GnRH administration present. According to Jeong (2010), the use of medications applied during pregnancy of women may negatively affect liver metabolism, which could explain the lower MY in the heifers of the study.

In our study, the MY progressively increased between the first three weeks of lactation, which was similar to what was observed in a study with induced lactating cows and heifers in a 12-day PIL and using as hormones PGF2α, estradiol, progesterone, reserpine, and dexamethasone (Ramgattie et al., 2014). After this period, MY in the present study increased at a slower rate, until reaching the maximum production (peak lactation) between the 11th week of lactation, contrasting that found in primiparous with natural lactation, where the peak of MY occurs in the 6th week of lactation (Bjelland et al., 2011). However, the mechanism that causes lactating-induced cows and heifers to delay reaching the peak of MY is not well understood (Mellado et al., 2011). One explanation for the delay in reaching the peak of MY could be because the exogenous hormones injectable during LIP lead to an incomplete stimulus in the development of the mammary gland and alveoli, delaying the moment of peak milk production.

The heifers in the present study produced an average of 16.22 ± 1.04 kg/milk/day at peak production. However, the MY results were lower than the studies by Macrina et al. (2011b, 2014), who described MY of 22.7 kg/milk/day in 15-month old Holstein heifers and 26.1 kg/milk/day and 22.9 kg/milk/day in Holstein heifers aged 18 and 14 months, respectively. The difference in the peak on MY found in the present study with the others may be linked to the different breed and animal genetics, since, according to some studies, it is expected that animals of the Holstein breed produce more milk when compared to their crossbreeds (Holstein x Jersey) as used in the present study (Bjelland et al., 2011).

Milk composition

The averages of fat (4.74 ± 0.22%), protein (3.55 ± 0.14%), lactose (4.48 ± 0.12%), TS (13.8 ± 0.31%) and MSNF (9.03 ± 0.18%) during 90 DIM were similar to milk components for cows and primiparous with conventional lactations. Auldist et al. (2007) described levels of 4.04% fat and 3.40% protein in cows, similar to the results reported in the present study. However, other studies have described different milk compositions in both cows and/or lactating heifers. Heifers with induced lactations produced milk with 3.68 to 3.66% fat and 3.35 to 3.25% protein at 15 months of age (Macrina et al., 2011a, 2011b).

In the present study, higher milk solid contents (fat and protein) were superior compared to Macrina et al. (2011b), who described 4.13% fat and 3.58% protein in Holstein heifers with induced lactation. An explanation for the higher milk solid contents could be that heifers used in the present study were crossbreed Holstein x Jersey, with high solids production when compared to Holstein (Auldist et al., 2007). The higher solids production is potentially due to the lower volume of milk produced in

induced animals, as high milk yields tend to dilute milk components (Macrina et al., 2011b).

Somatic cell count

The SCC log during the first 90 DIM of the present study was lower in ATBS-treated heifers compared to control (CONT1). Decreased SCC indicates that treatment with ATBS reduced the intensity of inflammation and improved mammary gland health with induced lactations. This effect was a result of the combined effect of curing IMI due to the use of injectable antibiotics and the prevention of new IMI (NIMI) with the ITS.

Similarly, other studies have also observed decreased SCC in heifers treated with antibiotics before calving using different antimicrobials: intramammary cephapirin (Oliver et al., 2003), intramammary cloxacillin (Sampimon et al., 2009), injectable penethamate hydriodide (Passchyn et al., 2013), and the combination of intramammary antibiotic and ITS (Machado & Bicalho, 2018), which indicated the effectiveness of these antimicrobials for curing subclinical mastitis.

Prevalence of IMI and pathogen distribution isolated from mastitis cases

Few studies used injectable antibiotic therapy in heifers (Naqvi et al., 2018), and there are no reports on the use of norfloxacin associated with teat sealant. According to our results, treatment with ATBS before the initiation of lactation reduced the prevalence of IMI in the mammary quarters during the first two weeks of lactation relative to the control group (CONT1). These results are similar to those described in studies evaluating IMI prevalence in primiparous cows with conventional lactations (De Vliegher et al., 2012; Fox, 2009). Similarly to the present study, a decrease in the prevalence of IMI was observed after treatment in heifers with intramammary antibiotics (Oliver et al., 2003), injectable antibiotics (Passchyn et al., 2013), and the association of teat sealant with intramammary antibiotics (Machado & Bicalho, 2018). Therefore, these results suggest that antibiotic treatment in heifers reduces IMI at the beginning of lactation, both in animals with induced and conventional lactations.

Heifers, such as those selected in the present study, have higher frequencies of pathogen isolation in early lactation collections, as these animals have a higher risk period for acquiring IMI when compared to younger heifers (De Vliegher et al., 2004). Our results showed that the most frequently isolated pathogens during the first two weeks of lactation were CNS and *S. aureus,* similar to that found in other studies of conventional lactating heifers, in which CNS was the most frequently isolated group of pathogens during the postpartum (De Vliegher et al., 2012; Machado & Bicalho, 2018). The effects of NIMI caused by CNS on MY are limited or even absent (Compton et al., 2007). The limited effect of CNS on decreasing MY may be linked to the present study, in which heifers of CONT1 treatment (without ATBS) showed higher frequencies in the isolation of SCN, and yet did not show lower MY compared to ATBS-treated heifers.

Mammary quarters that received ATBS treatment had a lower risk of IMI than the control group in the first two weeks of lactation. This result is a consequence of the injectable antibiotic in curing existing IMI cases and reducing the prevalence of IMI in early lactation. Furthermore, studies showed that injectable antibiotics may have clinical advantages over intramammary infusions such as greater diffusion of antibiotics when the intramammary route is compromised or lower risk for operators in infusing antibiotics (McDougall et al., 2007). Also, the use of sealant may have caused a positive effect due to the formation of a physical barrier in the cistern, which prevented pathogens from entering the GM (Freu et al., 2020). According to other studies, the use of teat sealant in heifers with conventional lactations decreases the risk of clinical and subclinical mastitis by 41 to 84% (Parker et al., 2007). Differently from the present study, the combination of teat sealant (2.6 bismuth subnitrate) and injectable antibiotic (tylosin) before calving in heifers did not decrease the prevalence of IMI (Parker et al., 2008). However, Parker et al. (2008) observed that in the mammary quarters treated only with teat sealant there was a lower prevalence and risk of IMI. Another study using teat sealant associated or not with intramammary amoxicillin in Holstein heifers found that the protocol with the antibiotic plus teat sealant association reduced the SCC and the incidence of MC and MSC when compared to animals treated only with antibiotics or teat sealant (Machado & Bicalho, 2018). Despite observing effects with the association of injectable antibiotics and ITS, our study has some limitations such as not evaluating the isolated effects of components.

Follicular cysts and return to cyclicity

Among the heifers that were LIP treated and submitted to the reproductive status evaluation at 15 DIM, 33.01% (n = 35/106) had FCs. Previous studies reported the occurrence of FCs in cows and heifers after the LIP (Freitas et al., 2010). The occurrence of FCs may be a response to the use of high hormonal dosages (estrogen and progesterone) applied

during treatment or by some endocrine imbalance of the hypothalamic-pituitary axis (Cook et al., 1990).

Heifers treated with ATBS had a greater cyclicity at 45 DIM than those from CONT1 (68% *vs* 35%). This result may have been due to lower SCC and lower prevalence of IMI, as a higher SCC may affect the reproductive efficiency of cows and heifers. On the other hand, the GnRH treatment did not affect FC luteinization and return to cyclicity at 45 DIM compared to untreated (CONT2; 57% vs 46%). Different results from the present study were reported for the resolution of FCs after lactation induction by use of GnRH alone (100 μg; Jordan et al., 1981) or associated with prostaglandin (PGF2α; Freitas et al., 2010). There was a high rate of spontaneous FC regression in heifers and, as a result, no effect of GnRH was observed. According to Garverick (1997), most FCs regress spontaneously over time and are replaced by new follicular waves, although the mechanisms leading to spontaneous regression of FCs are still unknown.

First-service conception rate and cumulative pregnancy rate

The average conception rate among the experimental groups at 120 days of gestation was 57.5% since 53 heifers were diagnosed as pregnant. Comparable results at first conception were found in non-LIP heifers: 61.3% in Holstein heifers, 51.1% in Jersey heifers, and 54.7% in heifers both aged from 13 to 15 months (Xu & Burton, 1999). Therefore, the conception rate observed after the LIP was similar in heifers of the same racial group that was not submitted to the LIP, which indicates that the LIP did not negatively influence the conception rate.

The average cumulative pregnancy rate (heifers that were inseminated and conceived within a period) assessed at 200 DIM regardless of treatment was 75.35%, which can be considered high due to the animals used in the case of heifers with induced lactations, and who had FCs in the beginning lactation. Also, this conception rate is similar to that described for heifers not submitted to LIP in other studies: 64.2% with Jersey heifers, 71.8% Holstein, and 70.4% Holstein × Jersey (Xu & Burton, 1999), 68% for Holstein heifers (Macmillan et al., 2017). These results suggest that the present LIP did not affect the reproductive performance of heifers when compared to uninduced heifers.

The use of GnRH for the treatment of FCs did not influence the final conception rate. However, the ATBS treatment negatively affected the final conception rate. This was an unexpected result mainly because it was the experimental group with a lower prevalence of IMI and lower SCC, and it was expected that heifers with better udder health have better reproductive efficiency, because intramammary infections can negatively influence reproduction. Machado & Bicalho (2018) reported that treatment with antibiotic and teat sealant did not influence the reproductive performance of heifers. Moreover, the occurrence of FCs at the beginning of lactation did not influence the final conception rate, similar to that observed with Holstein cows that presented FCs after LIP (Freitas et al., 2010).

The present study has the limitation of not comparing data on milk production and composition between animals with induced and conventional lactations. This occurred because all animals used in the study had their lactations induced. Additionally, the blood collections were not conducted, which would generate interesting results on the serum and hormonal profile in the organism of heifers with induced lactation.

Conclusion

The LIP was efficient in inducing MY in Holstein x Jersey heifers changing the milk composition. Treatment with injectable norfloxacin associated with ITS reduced the IMI prevalence of IMI in the first 14 DIM and decreased the SCC. Also, the use of GnRH did not affect the FC regression, cyclicity at 45 DIM, and cumulative pregnancy rates.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

The experimental procedures were performed according to the ethical principles of animal experimentation and were approved by the Faculty of Veterinary Medicine and Animal Science (FMVZ) Animal Use Ethics Committee (CEUA - nº 8212170616).

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References

Al-Katanani YM, Webb DW, Hansen PJ. Factors affecting seasonal variation in 90-day nonreturn rate to first service in lactating Holstein cows in a hot climate. J Dairy Sci. 1999;82(12):2611-6. http://dx.doi.org/10.3168/jds.S0022-0302(99)75516-5. PMid:10629807.

Auldist MJ, Pyman MFS, Grainger C, Macmillan KL. Comparative reproductive performance and early lactation productivity of Jersey × Holstein cows in predominantly Holstein herds in a pasture-based dairying system. J Dairy Sci. 2007;90(10):4856-62. http://dx.doi.org/10.3168/jds.2006-869. PMid:17881709.

Bjelland DW, Weigel KA, Hoffman PC, Esser NM, Coblentz WK, Halbach TJ. Production, reproduction, health, and growth traits in backcross Holstein × Jersey cows and their Holstein contemporaries1. J Dairy Sci. 2011;94(10):5194-203. http://dx.doi.org/10.3168/jds.2011-4300. PMid:21943769.

Borm AA, Fox LK, Leslie KE, Hogan JS, Andrew SM, Moyes KM, Oliver SP, Schukken YH, Hancock DD, Gaskins CT, Owens WE, Norman C. Effects of prepartum intramammary antibiotic therapy on udder health, milk production, and reproductive performance in dairy heifers. J Dairy Sci. 2006;89(6):2090-8. http://dx.doi.org/10.3168/jds.S0022-0302(06)72279-2. PMid:16702275.

Byatt JC, Sorbet RH, Eppard PJ, Curran TL, Curran DF, Collier RJ. The effect of recombinant bovine placental lactogen on induced lactation in dairy heifers. J Dairy Sci. 1997;80(3):496-503. http://dx.doi.org/10.3168/jds.S0022-0302(97)75962-9. PMid:9098799.

Compton CWR, Heuer C, Parker K, McDougall S. Epidemiology of mastitis in pasture-grazed peripartum dairy heifers and its effects on productivity. J Dairy Sci. 2007;90(9):4157-70. http://dx.doi.org/10.3168/jds.2006-880. PMid:17699034.

Cook DL, Smith CA, Parfet JR, Youngquist RS, Brown EM, Garverick HA. Fate and turnover rate of ovarian follicular cysts in dairy cattle. J Reprod Fertil. 1990;90(1):37-46. http://dx.doi.org/10.1530/jrf.0.0900037. PMid:2121974.

De Vliegher S, Fox LK, Piepers S, McDougall S, Barkema HW. Invited review: mastitis in dairy heifers: nature of the disease, potential impact, prevention, and control. J Dairy Sci. 2012;95(3):1025-40. http://dx.doi.org/10.3168/jds.2010-4074. PMid:22365187.

De Vliegher S, Laevens H, Barkema HW, Dohoo IR, Stryhn H, Opsomer G, Kruif A. Management practices and heifer characteristics associated with early lactation somatic cell count of Belgian dairy heifers. J Dairy Sci. 2004;87(4):937-47. http://dx.doi.org/10.3168/jds.S0022-0302(04)73238-5. PMid:15259228.

Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. A body condition scoring chart for holstein dairy cows. J Dairy Sci. 1989;72(1):68-78. http://dx.doi.org/10.3168/jds.S0022-0302(89)79081-0.

Fox LK. Prevalence, incidence and risk factors of heifer mastitis. Vet Microbiol. 2009;134(1–2):82-8. http://dx.doi.org/10.1016/j.vetmic.2008.09.005. PMid:18930610.

Freitas PRC, Coelho SG, Rabelo E, Lana ÂMQ, Artunduaga MAT, Saturnino HM. Artificial induction of lactation in cattle. Rev Bras Zootec. 2010;39(10):2268-72. http://dx.doi.org/10.1590/S1516-35982010001000024.

Freu G, Tomazi T, Monteiro CP, Barcelos MM, Alves BG, Santos MV. Internal teat sealant administered at drying off reduces intramammary infections during the dry and early lactation periods of dairy cows. Animals. 2020;10(9):1-16. http://dx.doi.org/10.3390/ani10091522. PMid:32872097.

Fulkerson WJ, McDowell GH. Artificial induction of lactation in cattle by use of dexamethasone trimethylacetate. Aust J Biol Sci. 1975;28(2):183-7. http://dx.doi.org/10.1071/BI9750183. PMid:1167131.

Fulkerson WJ. Artificial induction of lactation: a comparative study in heifers. Aust J Biol Sci. 1978;31(1):65-72. http://dx.doi.org/10.1071/BI9780065.

Garverick HA. Ovarian follicular cysts in dairy cows. J Dairy Sci. 1997;80(5):995-1004. http://dx.doi.org/10.3168/jds.S0022-0302(97)76025-9. PMid:9178141.

Harness JR, Anderson RR, Thompson LJ, Early DM, Younis AK. Induction of lactation by two techniques: success rate, milk composition, estrogen and progesterone in serum and milk, and ovarian effects. J Dairy Sci. 1978;61(12):1725-35. http://dx.doi.org/10.3168/jds.S0022-0302(78)83794-1. PMid:744808.

Jeong H. Altered drug metabolism during pregnancy: hormonal regulation of drug-metabolizing enzymes. Expert

Opin Drug Metab Toxicol. 2010;6(6):689-99. http://dx.doi.org/10.1517/17425251003677755. PMid:20367533.

Jordan DL, Erb RE, Malven PV, Callahan CJ, Veenhuizen EL. Artificial induction of lactation in cattle: effect of modified treatments on milk yield, fertility, and hormones in blood plasma and milk. Theriogenology. 1981;16(3):315-29. http://dx.doi.org/10.1016/0093-691X(81)90016-9. PMid:16725644.

Kreiger M, Friton GM, Hofer J, Fuchs K, Winter P. Effects of periparturient systemic treatment with penethamate hydriodide on udder health and milk yield of dairy heifers. J Dairy Res. 2007;74(4):392-8. http://dx.doi.org/10.1017/S0022029907002683. PMid:17651515.

Lakhani P, Thakur A, Kumar S, Singh P. Artificial induction of lactation in bovines: scope and limitations. Int J Livest Res. 2017;1(7):102-12. http://dx.doi.org/10.5455/ijlr.20170324031735.

Lucy MC. Reproductive loss in high-producing dairy cattle: where will it end? J Dairy Sci. 2001;84(6):1277-93. http://dx.doi.org/10.3168/jds.S0022-0302(01)70158-0. PMid:11417685.

Machado VS, Bicalho RC. Prepartum application of internal teat sealant or intramammary amoxicillin on dairy heifers: effect on udder health, survival, and performance. J Dairy Sci. 2018;101(2):1388-402. http://dx.doi.org/10.3168/jds.2017-13415. PMid:29224874.

Macmillan K, Loree K, Mapletoft RJ, Colazo MG. Short communication: optimization of a timed artificial insemination program for reproductive management of heifers in Canadian dairy herds. J Dairy Sci. 2017;100(5):4134-8. http://dx.doi.org/10.3168/jds.2016-12318. PMid:28237601.

Macrina AL, Kauf ACW, Kensinger RS. Effect of bovine somatotropin administration during induction of lactation in 15-month-old heifers on production and health. J Dairy Sci. 2011a;94(9):4566-73. http://dx.doi.org/10.3168/jds.2011-4394. PMid:21854929.

Macrina AL, Kauf ACW, Pape-Zambito DA, Kensinger RS. Induced lactation in heifers: effects of dexamethasone and age at induction on milk yield and composition. J Dairy Sci. 2014;97(3):1446-53. http://dx.doi.org/10.3168/jds.2013-7241. PMid:24440271.

Macrina AL, Tozer PR, Kensinger RS. Induced lactation in pubertal heifers: efficacy, response to bovine somatotropin, and profitability. J Dairy Sci. 2011b;94(3):1355-64. http://dx.doi.org/10.3168/jds.2010-3811. PMid:21338801.

McDougall S, Agnew KE, Cursons R, Hou XX, Compton CRW. Parenteral treatment of clinical mastitis with tylosin base or penethamate hydriodide in dairy cattle. J Dairy Sci. 2007;90(2):779-89. http://dx.doi.org/10.3168/jds.S0022-0302(07)71562-X. PMid:17235155.

Mellado M, Antonio-Chirino E, Meza-Herrera C, Veliz FG, Arevalo JR, Mellado J, Santiago A. Effect of lactation number, year, and season of initiation of lactation on milk yield of cows hormonally induced into lactation and treated with recombinant bovine somatotropin. J Dairy Sci. 2011;94(9):4524-30. http://dx.doi.org/10.3168/jds.2011-4152. PMid:21854924.

Mellado M, Nazarre E, Olivares L, Pastor F, Estrada A. Milk production and reproductive performance of cows induced into lactation and treated with bovine somatotropin. Anim Sci. 2006;82(4):555-9. http://dx.doi.org/10.1079/ASC200656.

Middleton JR, Timms LL, Bader GR, Lakritz J, Luby CD, Steevens BJ. Effect of prepartum intramammary treatment with pirlimycin hydrochloride on prevalence of early first-lactation mastitis in dairy heifers. J Am Vet Med Assoc. 2005;227(12):1969-74. http://dx.doi.org/10.2460/javma.2005.227.1969. PMid:16379636.

Moussavi HA, Mesgaran MD, Gilbert RO. Effect of mastitis during the first lactation on production and reproduction performance of Holstein cows. Trop Anim Health Prod. 2012;44(7):1567-73. http://dx.doi.org/10.1007/s11250-012-0107-3. PMid:22395793.

Naqvi SA, De Buck J, Dufour S, Barkema HW. Udder health in Canadian dairy heifers during early lactation. J Dairy Sci. 2018;101(4):3233-47. http://dx.doi.org/10.3168/jds.2017-13579. PMid:29397171.

National Mastitis Council - NMC. Laboratory handbook on bovine mastitis. New Prague: National Mastitis Council; 1999.

National Research Council - NRC. Nutrient requeriments of dairy cattle. 7th ed. Washington, DC: The National Academies Press; 2001. 381 p.

Oliver SP, Lewis MJ, Gillespie BE, Dowlen HH, Jaenicke EC, Roberts RK. Prepartum antibiotic treatment of heifers: milk production, milk quality and economic benefit. J

Dairy Sci. 2003;86(4):1187-93. http://dx.doi.org/10.3168/jds.S0022-0302(03)73702-3. PMid:12741543.

Parker KI, Compton C, Anniss FM, Weir A, Heuer C, McDougall S. Subclinical and clinical mastitis in heifers following the use of a teat sealant precalving. J Dairy Sci. 2007;90(1):207-18. http://dx.doi.org/10.3168/jds.S0022-0302(07)72622-X. PMid:17183089.

Parker KI, Compton CWR, Anniss FM, Heuer C, McDougall S. Quarter-level analysis of subclinical and clinical mastitis in primiparous heifers following the use of a teat sealant or an injectable antibiotic, or both, precalving. J Dairy Sci. 2008;91(1):169-81. http://dx.doi.org/10.3168/jds.2007-0212. PMid:18096938.

Passchyn P, Piepers S, De Vliegher S. Systemic prepartum treatment of end-term dairy heifers with penethamate hydriodide: effect on udder health, milk yield, and culling until 120 days in milk. J Dairy Sci. 2013;96(10):6324-35. http://dx.doi.org/10.3168/jds.2013-6626. PMid:23932138.

Ramgattie R, Siew N, Diptee M, Stoute V, Knights M. Effect of mammary stimulation on dairy cows and heifers exposed to a lactation induction protocol. Open J Anim Sci. 2014;1(4):1-12. http://dx.doi.org/10.4236/ojas.2014.41001.

Sampimon OC, De Vliegher S, Barkema HW, Sol J, Lam TJGM. Effect of prepartum dry cow antibiotic treatment in dairy heifers on udder health and milk production. J Dairy Sci. 2009;92(9):4395-403. http://dx.doi.org/10.3168/jds.2009-2203. PMid:19700699.

Schrick FN, Hockett ME, Saxton AM, Lewis MJ, Dowlen HH, Oliver SP. Influence of subclinical mastitis during early lactation on reproductive parameters. J Dairy Sci. 2001;84(6):1407-12. http://dx.doi.org/10.3168/jds.S0022-0302(01)70172-5. PMid:11417699.

Schukken YH, Wilson DJ, Welcome F, Garrison-Tikofsky L, Gonzalez RN. Monitoring udder health and milk quality using somatic cell counts. Vet Res. 2003;34(5):579-96. http://dx.doi.org/10.1051/vetres:2003028. PMid:14556696.

Sejrsen K, Purup S. Influence of prepubertal feeding level on milk yield potential of dairy heifers: a review. J Anim Sci. 1997;75(3):828-35. http://dx.doi.org/10.2527/1997.753828x. PMid:9078503.

Smith KL, Schanbacher FL. Hormone induced lactation in the bovine. I. Lactational performance following injections of 17β-estradiol and progesterone. J Dairy Sci. 1973;56(6):738-43. http://dx.doi.org/10.3168/jds.S0022-0302(73)85243-9. PMid:4708130.

Smith KL, Schanbacher FL. Hormone induced lactation in the bovine. II. Response of nulligravida heifers to modified estrogen-progesterone treatment1. J Dairy Sci. 1974;57(3):296-303. http://dx.doi.org/10.3168/jds.S0022-0302(74)84880-0.

Socha MT, Johnson AB. Dietary recommendations for reclacement heifers. Kraftfutter (Frankf). 2000;4:156-60.

Trinidad P, Nickerson SC, Luther DG. Antimicrobial susceptibilities of staphylococcal species isolated from mammary glands of unbred and primigravid dairy heifers. J Dairy Sci. 1990;73(2):357-62. http://dx.doi.org/10.3168/jds.S0022-0302(90)78681-X. PMid:2329202.

Van Amburgh ME, Fox DG, Galton DM, Bauman DE, Chase LE. Evaluation of national research council and Cornell net carbohydrate and protein systems for predicting requirements of Holstein heifers. J Dairy Sci. 1998;81(2):509-26. http://dx.doi.org/10.3168/jds.S0022-0302(98)75603-6. PMid:9532506.

Xu ZZ, Burton LJ. Reproductive performance of dairy heifers after estrus synchronization and fixed-time artificial insemination. J Dairy Sci. 1999;82(5):910-7. http://dx.doi.org/10.3168/jds.S0022-0302(99)75309-9. PMid:10342228.

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