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Influence of the reuse of progesterone implants in a fixed-time artificial insemination protocol on the conception rates of lactating cows during two different seasons

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This study aimed to evaluate the influence of reusing progesterone intravaginal implants using a fixed-time artificial insemination (FTAI) protocol on the conception rates of 593 primiparous and multiparous lactating cows during two different seasons. The cows were divided into two categories: multiparous and primiparous. The experiment was conducted during seasons with high and mild temperatures. To compare the conception rates during both seasons, the animals were randomly divided into three groups according to use progesterone implant (1st use, 2nd use and 3rd use) on the first day of the synchronization protocol for FTAI within each category. A pregnancy diagnosis was performed at 30 days following FTAI. The temperature and humidity index was higher (P<0.01) during the season with higher temperatures (74.4 ± 0.26) than during the season with mild temperatures (67.5 ± 0.34). The different categories (multiparous and primiparous) did not influence the conception rate in terms of the seasonal temperatures or the reuse of progesterone implants. Nevertheless, a 3rd use implant administered during the season with high temperatures was 1.98 times less likely to result in a pregnancy compared with a 1st use implant (P=0.01) and 2.83 less likely to result in a pregnancy than the other implants used during the season with mild temperatures (P=0.005). The results indicate that during the season with mild temperatures, the conception rates at 30 days are not influenced by reusing the P_4 implant in any of the categories analyzed. However, during the season with high temperatures, the reuse of the P_4 implant can influence the conception rates of dairy cows.

Key words: Heat stress, Holstein cows, pregnancy.

INTRODUCTION

The increasing demand for animals with higher milk productivity has intensified genetic breeding of herds; however, there has also been a decrease in the reproductive efficiency of these cows (Lucy, 2001). To meet this production requirement, there is a need for a high dry matter intake, which promotes an increase in hepatic blood flow and in the rate of steroid hormone metabolism, thus decreasing blood concentrations of

steroids, which can negatively affect reproductive function (Sangsritavong et al., 2002). The progesterone is involved in oocyte quality, follicular dominance, embryonic growth and maintenance of pregnancy (Leonhardt and Edwards, 2002). Low pre-insemination progesterone levels are responsible for the persistence of dominant follicles, producing a low quality oocyte upon ovulation (Bisinotto et al., 2013). Mann and Lamming (2001) reported that embryonic development is impaired when there are low levels of progesterone exposure following conception due to the low levels of interferon-tau release and, therefore, prostaglandin $F_{2\alpha}$ release. Reproductive challenges increase with heat stress, reproductive diseases, inadequate nutrition, and increased animal productivity. The problem of reproductive inefficiency in high producing dairy cows may be minimized with genetic selection and breeding (Banos et al., 2013). All these factors have leveraged the use of reproductive biotechnologies as artificial insemination, multiple ovulation, *in vitro* fertilization and cloning to optimize the production and reproductive performance of the herd in a more rational and economical way (Binelli et al., 2009). Thus, fixed-time artificial insemination (FTAI) has helped in reducing the number of days in lactation until the first insemination and the calving interval in dairy herds (Mapletoft et al., 2009).

Even though others reports have compared the progesterone concentration achieved after insertion of new or reused progesterone implants (Zuluaga and Williams, 2008; Abdallah and El Rahim, 2014). However, there are no previous reports of the association of reusing progesterone implants in different categories of dairy cows and seasons. This study aimed to determine the influence of reusing progesterone implants in FTAI protocols on the conception rate in primiparous and multiparous lactating cows during seasons with high temperatures and mild temperatures.

MATERIALS AND METHODS

Location

The experiment was conducted on a dairy farm in the city of Montividiu in southwestern Goiás State, Brazil (latitude 17°20'5.7" and longitude 51°18'46.7"). During the experiment, lactating Holstein cows were confined in wooded feedlots and supplied with drinking troughs. The animals received a complete diet consisting of quality corn silage and a concentrated diet balanced for milk production (NRC, 2001), which was distributed four times a day using a mixing wagon.

Experimental procedures

The ambient temperature and relative humidity were measured daily throughout the experimental period. During the season with mild temperatures (May to August 2012), the average temperature was 21.1 ± 1.3°C, with a maximum of 23.4 ± 1.2°C and a minimum of 18.9 ± 1.5°C; the average relative humidity was 63 ± 14%. During the season with high temperatures (September 2012 to April 2013), the average temperature was 24.8 ± 0.7°C, with a maximum of 28.3 ± 0.6°C and a minimum of 20.1 ± 0.5°C; the average relative humidity was 78 ± 2%. Heat stress was indicated by the temperature-humidity index (THI) for each period, which was calculated using the following formula used by Mader et al. (2006): THI = T x 0.8 + [(RH (%) ÷ 100 x (T - 14.4)] + 46.4, where T is the temperature in degrees Celsius and RH is the relative humidity of the air. The mean and standard error of the THI was 67.5 ± 0.34 for the season with mild temperatures and 74.4 ± 0.26 for the season with high temperatures.

Animals

The experimental animals (n = 593) were divided into two categories: multiparous (mean of 28.7 ± 3.6 kg milk.day^{-1} [n = 342]) and primiparous (mean of 25 ± 4.3 kg milk.day^{-1} [n = 251]). The selected cows were 58.7 ± 9.3 days postpartum; had a body condition score between 2.5 and 3.5 on a scale from 1 to 5, with 1 being very thin and 5 being very fat (Ferguson et al., 1994); and had a uterus without signs of infection upon clinical examination and ultrasonography (Mindray® DP3300 VET).

Experimental groups

To compare the conception rates of each category during both seasons, the animals were randomly divided into three groups according to use progesterone implant (1st use, 2nd use and 3rd use) on the first day of the fixed-time artificial insemination protocol.

Hormonal treatment

The synchronization of follicular wave started on the first day of the fixed-time artificial insemination protocol (D0), when the animals in each group received a 1st, 2nd, or 3rd use intravaginal progesterone implant (Cronipres®, Biogénesis-Bagó, Garin, province of Buenos Aires, Argentina) and intramuscular application of 2 mg of estradiol benzoate (Bioestrogen ®, Biogenesis-Bagó, Garin, province of Buenos Aires, Argentina). After eight days (D8), the implant was removed, and 0.15 mg of sodium cloprostenol (Bioestrogen®, Biogénesis-Bagó, Garin, province of Buenos Aires, Argentina), 300 IU of equine chorionic gonadotropin (Folligon®, Intervet International B.V., Boxmeer, Holland), and 1 mg of estradiol cypionate were administered (ECP®, Pfizer, Pharmacia and Upjohn Company, NY, USA). Forty-eight hours after implant removal (D10), 0.004 mg of buserelin acetate (Sincroforte®, Ouro Fino, Cravinhos, SP, Brazil) was administered intramuscularly. The artificial insemination was performed with semen from the same bull and single inseminator. A pregnancy diagnosis was performed at 30 days following FTAI using an ultrasound device equipped with a 5.0 MHz linear transducer (Mindray® DP3300 VET).

Statistical analysis

The experimental design was completely randomized using a 2 x 2

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Table 1. Conception rates at 30 days in lactating cows according to the category (multiparous and primiparous), reuse of progesterone implants (1st, 2nd, and 3rd use) in the FTAI protocol, and seasons with mild and high temperatures.

Season	Implant	Conception rate at 30 days	
		Multiparous	Primiparous
Mild temperatures	1st use	40.0% (10/25)	54.5% (6/11)
	2nd use	43.9% (25/57)	55.0% (11/20)
	3rd use	50.0% (14/28)	52.6% (10/19)
High temperatures	1st use	39.3% (57/145)	46.3% (50/108)
	2nd use	34.6% (18/52)	41.0% (16/39)
	3rd use	28.6% (10/35)	25.9% (14/54)

Table 2. Analysis of deviance for the conception rate at 30 days in lactating cows in a factorial design for the different seasons, progesterone implants and categories.

Variable	DF	Deviance	ΔDF	Δ Deviance	P value
Null			592	801.17	-
Season	1	4.2373	591	796.93	0.03954
Implant	2	3.6727	589	793.26	0.15940
Category	1	1.9182	588	791.34	0.16606
Season : implant	2	3.6636	586	787.68	0.05012
Implant : category	2	0.8949	583	786.64	0.63924
Season : implant : category	2	0.0154	581	786.63	0.99234

x 3 factorial design to evaluate the effect of two categories (multiparous and primiparous), two seasons (high and mild temperatures), and three treatments (1st, 2nd, and 3rd use implants). The data of conception rate were analyzed using multiple logistic regression with a fixed regression model, considering a significance level of 5%. The model included the effects of treatment, season, implant and THI; because, it is unbalanced data, the variables and interactions between significant variables in the analysis of deviance ($P<0.05$) were considered in the final model. The odds ratios and their respective confidence intervals at 95% probability were calculated for the variables considered in the final model. The program R software (R Core Team, 2014) was used to perform the analysis.

RESULTS

The THI was higher ($P<0.01$) during the season with the high temperatures (74.4 ± 0.26) than during the season with the mild temperatures (67.5 ± 0.34). The conception rates at 30 days for multiparous and primiparous cows according to the progesterone implant used in the FTAI protocol during the seasons of high and mild temperatures are listed in Table 1.

The analysis of deviance for the conception rate at 30 days in lactating cows is presented in Table 2. The season of the year was a significant effect in the concept rate ($P=0.03954$). The different categories (primiparous and multiparous) and the reuse of progesterone implants did not influence the conception rates.

However, there was a significant interaction of the reuse of progesterone implants and the season of the year with respect to the conception rate (Table 2). The logistic regression model applied to the variables that showed a significant interaction to conception rate of lactating cows in the analysis of deviance is presented in Table 3.

According to the logistic regression model, a 3rd use implant administered during the season with high temperatures was 1.98 times less likely to result in a pregnancy compared with a 1st use implant ($P=0.01$) and 2.83 times less likely to result in a pregnancy than the other implants used during the season with mild temperatures ($P=0.005$).

DISCUSSION

In both categories, there were no statistically significant differences in the conception rate with the reuse of 1st, 2nd, and 3rd use implants during the season with mild temperatures. These results are consistent with those observed by Mendonça et al. (2012), who reported no significant differences in the conception rates of

Table 3. Logistic regression for the conception rate of lactating cows following FTAI with 1st, 2nd, and 3rd use progesterone implants during seasons with high and mild temperatures.

Variable	Factor	Odds ratio	Confidence interval (95%)	P value
Implant - 1st use	-	1.00	-	-
Implant - 2nd use	Mild temperatures	0.91	0.41 - 2.02	0.82
	High temperatures	- 1.23	0.75 - 2.01	0.41
Implant - 3rd use	Mild temperatures	0.77	0.32 - 1.83	0.55
	High temperatures	- 1.98	1.17 - 3.37	0.01
Mild season	-	1.00	-	-
Hot season	Implant - 1st use	1.09	0.54 - 2.21	0.81
	Implant - 2nd use	- 1.47	0.79 - 2.73	0.22
	Implant - 3rd use	- 2.83	1.35 - 5.92	0.005

crossbred dairy cows when implants containing 1.9 g of progesterone were reused in an FTAI protocol. Rocha et al. (2007) also found no difference in the conception rates of Nellore cows upon the reuse of implants containing one gram of progesterone for up to four times. Guido et al. (2008) reported similar conception rates in lactating goats synchronized with new and used progesterone implants (53.3 versus 46.7%). High-producing dairy cows ingest diets that are denser, have an increased hepatic blood flow, and consequently exhibit an increase rate of steroid hormone metabolism that leads to a decrease in blood progesterone concentrations (Sangsritavong et al., 2002). Therefore, the implants should be reused with caution during periods with high temperatures, which can reduce the conception rates because of the metabolic obstacles to which cows are subjected. Climate factors are highly relevant for conception rate (Garcia-Ispierto et al., 2007). The THI during the season with the high temperatures had effect in conception rate of lactating dairy cows. According to Wheelock et al. (2010), values below 65 are within the range of thermal comfort for dairy cows. The action of heat stress on the follicle tends to produce oocytes with a lower fertilization capacity, and if this process occurs, the embryo does not develop normally (Hansen, 2002). In this study, 3rd use implants were unable to maintain similar conception rates in the different categories between the seasons evaluated (Table 3). This finding may be due to heat stress observed during the season with the high temperatures, reducing the concentrations of plasma progesterone necessary for recognizing and maintaining a pregnancy. According to Barbosa et al. (2011), the conception rate during the fall/winter (42.55%) is higher than the conception rate during the spring/summer (25%) when using implants containing 1.9 g of progesterone in an FTAI protocol. However, this difference was not observed when they compared the reuse of implants. Nevertheless, Barbosa et al. (2011) found no difference in the conception rates of crossbred dairy cows in the presence or absence of a corpus luteum at the beginning of the FTAI protocol.

Conclusion

The reuse of progesterone implants in FTAI protocols is an alternative for achieving good reproductive rates in dairy cows. However, the implants should be reused with caution during periods with high temperatures. As a result of non-pregnancy, mismanagement can lead to an increase in the calving-to-conception interval and in the calving interval.

Conflict of interests

The author(s) have not declared any conflict of interests.

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