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RESEARCH ARTICLE

Effects of reproductive tract development and hormonal treatment on estrous synchronization in heifers

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ABSTRACT

Evaluation of the reproductive tract development (RTD) is a criterion rarely used when heifers are included in estrous synchronization programs. The objective of the present study was to determine the effect of RTD (mature and immature) and hormonal treatment (EC and EB) on estrous expression rate and pregnancy rate in *Bos indicus* heifers under tropical conditions. RTD was evaluated with an ultrasound and classified as mature (n = 99) or immature (n = 101). Heifers received an intravaginal device (DIB®; day 0), 2 mg of estradiol benzoate (EB) and 250 μg of cloprostenol ($PGF_2\alpha$). The DIB was removed on day 7 post insertion, and 250 μg of $PGF_2\alpha$ was applied and heifers were divided into two groups: One group of heifers received 0.5 mg of estradiol cypionate (EC group), and on day 8, a second group of heifers received 1 mg of estradiol benzoate (EB group). Estrus was detected by visual observation, and all heifers were inseminated at a fixed-time (FTAI). Fifteen days after insemination, bulls were introduced to the treatment groups. Data were analyzed using general modeling and binary logistic regression procedures. The percentage of estrus was similar for both mature and immature heifers ($P > 0.05$), however, the heifers treated with EB had 17% animals in estrus, than the heifers that received EC ($P < 0.05$). The pregnancy rate after FTAI (74.8%) and total pregnancy (FTAI + natural mating = 91.9%) was greater for the group of heifers with a mature reproductive tract ($P < 0.05$). The hormone treatment had not effect ($P > 0.05$) on the pregnancy rate. In conclusion, the heifers with mature reproductive tracts had the highest pregnancy rate after artificial insemination and total pregnancy. Hormonal treatment did not influence the pregnancy rate.

Keywords: *Bos indicus*; Estradiol benzoate; Estradiol cypionate; Pregnancy rate; Tropics.

INTRODUCTION

In the bovine production systems under tropical conditions, the most common criteria for replacement of heifers are body condition score, body weight, and age of heifer (Barcellos et al., 2014; Lardner et al., 2014). Evaluation of the reproductive tract is rarely applied, probably because it requires transrectal palpation of the internal genital organs to check the development of the uterus and ovaries. However, it has been shown to be a useful tool for decision making and tends to improve the breeding success of the herd by excluding heifers that probably cannot become pregnant during their first breeding season (Holm et al., 2009; Gutierrez et al., 2014; Dickinson et al., 2019).

In this sense, the evaluation of reproductive tract development (RTD) has been used to determine the age at puberty (Monteiro et al., 2013; Holm et al., 2015; Bruinjé et al., 2019), fertility in *postpartum* cows (Baez et al., 2016; Holm et al., 2016) and the response to estrus synchronization (Kasimanickam et al., 2016; Kasimanickam et al., 2020). Young et al. (2011) indicated that the size of the reproductive tract at the time of artificial insemination could be related to the fertility of dairy cattle.

Moreover, a high percentage of *Bos indicus* and cross beef heifers in fixed-time artificial insemination (FTAI) programs have been reported to have immature reproductive tracts (Claro et al., 2010; Kasimanickam et al., 2017). This was associated with a poor pregnancy rate because of the asynchrony between estrus and ovulation (Bó and Baruselli, 2014). One option to improve the synchrony between

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estrus and ovulation in synchronization programs is the application of valerate, cypionate or benzoate estradiol (Melo et al., 2016; Silva et al., 2018).

In this regard, Sales et al. (2012) report that EB and EC have different pharmacokinetic structures. The EC is the result of the esterification of estradiol by the propionic cyclopentane acid, which results in a low solubility in water and the consequent slower release of the administration site and a longer biological activity in comparison with EB (Vynckier et al., 1990), which acts faster than the EC. For this reason, the EC should be administered at the withdrawal of the progestogen and the EB must be administered 24 h after the withdrawal of the DIB, which requires an additional management for the animals, aspect that can stress the heifers and reduce the rate of pregnancy.

These hormones (EC and EB) have the function of regulating the follicular and luteal phases (Bó et al., 2016), and when the intravaginal device is removed, they have the function of standardizing and reducing the time of ovulation (Peralta-Torres et al., 2010; Sales et al., 2012), thus improving the pregnancy rate (Bó et al., 2013).

Information on the effects of RTD and hormonal treatment on estrous synchronization in zebu heifers under tropical conditions is scarce (Holm et al., 2016). Therefore, the objective of the present study was to determine the effect of RTD (mature and immature) and hormonal treatment (EC and EB) on estrous expression rate and pregnancy rate in *Bos indicus* heifers under tropical conditions. Our hypothesis was that *Bos indicus* heifers with reproductive tract mature would have higher pregnancy rate. A secondary hypothesis was that heifers received EC upon withdrawal from the intravaginal device are less stressed, which results in a higher pregnancy rate.

MATERIAL AND METHODS

The experiment was carried out in a ranch located in Tabasco, Mexico, between 17° 59' N and 92° 56' W at an altitude of 20 m. The climate of the region is humid tropical with an average temperature of 28.7 °C and an annual rainfall of 1,940.6 mm (INEGI, 2014).

Two hundred Brahman (*Bos indicus*) heifers with 30.5±1.23 months of age, body weight of 353.4±10.0 kg, and body condition score of 4.65±0.07 (Ayala et al., 1995) were considered. The heifers were kept, all day, in paddocks of Star grass (*Cynodon plectostachyus*) and given 1 kg of commercial feed/animal/day. The feed contained 14% crude protein. All study procedures were conducted according to the Mexican Official Standard NOM-062-ZOO-1999 for the production, care, and use of experimental animals.

At the start of the study (day 0), the RTD of the heifers was evaluated using a real-time ultrasound (Emperor®-830 Vet, with a 6.5 MHz linear transductor). The heifers were classified into two groups (mature and immature; Fig. 1), criteria for classifying the RTD of *Bos indicus* heifers were adapted from Anderson et al. (1991) and Cutaia et al. (2006), as described in Table 1.

Thereafter, an intravaginal device (DIB® Lab. Syntex, Argentina) containing 1 g of progesterone was inserted into each heifer, and 2 mg of estradiol benzoate (EB; Lab. Syntex, Argentina) and 250 μg of cloprostenol ($PGF_{2\alpha}$; Ciclase®, Lab. Syntex, Argentina) were intramuscular administered (Fig. 2). Seven days post-insertion, the DIBs were removed, and 250 μg $PGF_{2\alpha}$ was applied. Heifers with mature (n=99) or immature reproductive tracts (n=101) were further divided into two groups. The first group of heifers received 0.5 mg of estradiol cypionate (EC group; Lab. Syntex, Argentina) on the same day 7, and the second group received 1 mg of EB (EB group) 24 h later on day 8 (Peralta-Torres et al., 2010b and Butler et al., 2012; Fig. 2).

Twenty-four hours after removal of the DIBs, estrus was detected by visual observation over 60 h during 1-h intervals three times a day (06:00, 12:00, and 18:00 h). Fixed-time artificial insemination (FTAI) was carried out 52-56 h after the removal of the intravaginal device using frozen-thawed semen (Torres-Júnior et al., 2014).

Fifteen days after FTAI, proven bulls were introduced to heifers (1:30 ratio) for 30 d to determine the effect of reproductive synchronization on pregnancy. The pregnancy diagnosis was carried out using real-time ultrasound scanning. To establish differences between the pregnancy rate after FTAI and natural mating (NM), a first diagnosis was made 45 d after FTAI and a second 45 d after bull removal (day 90).

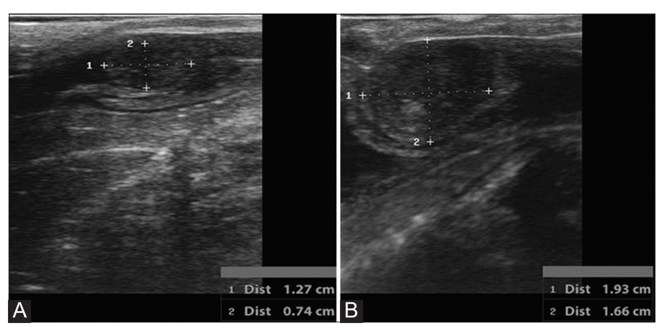

Fig 1. Reproductive tract development in Bos indicus heifers. (A) Immature. (B) Mature.

Table 1: Criteria for classifying reproductive tracts development of *Bos indicus* heifers

Reproductive tract development	Uterus		
	Diameter of the horn* (mm)	At the moment of palpation	Ovary structure (mm)
Immature	≤ 12.9	Without or with light tone	Follicle <10
Mature	≥ 13	With tone	Follicles >10 and presence of corpus luteum

* Cross-sectional measurement of the uterine horn, cranial to bifurcation

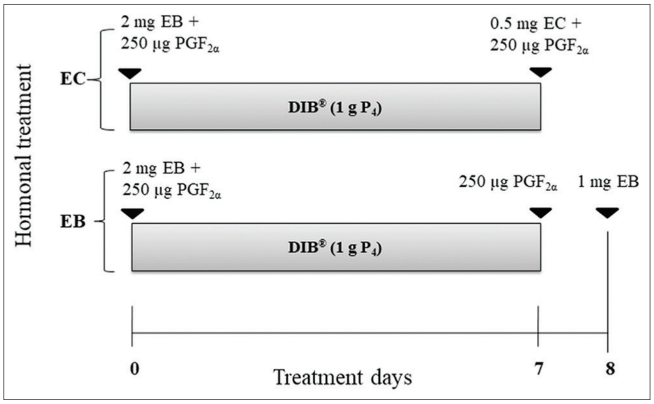

Fig 2. Synchronization program used in *Bos indicus* heifers with mature or immature reproductive tract. The following abbreviations were used: EC (estradiol cypionate), EB (estradiol benzoate), $PGF_{2\alpha}$ (prostaglandin $F_{2\alpha}$; cloprostenol), DIB® (intravaginal device), P_4 (progesterone).

The data of estrous presence (1 = Yes, 0 = Not), pregnancy status after FTAI (1 = Yes, 0 = Not), pregnancy status by NM (1 = Yes, 0 = Not) and total pregnancy (FTAI+NM) were subject to logistic regression analysis. Time data, from removal of the intravaginal device to estrus detection, was determined using general linear model analysis. Preliminary analysis of response variables included the fixed effects of RTD (mature or immature), hormonal treatment (EC or EB), and their interaction. However, interactions were not significant and were dropped from the final model.

Data on reproductive traits were described by a fixed binary logistic regression model:

$$\ln (p/(1-p)) = b_0 + b_1{*}RTDi + b_2{*}HTj$$

where: ln = natural logarithm; p = probability of the presence of estrous or pregnancy for a given heifer; b_0 = intercept; b_1 and b_2 = regression coefficients; RTD = immature or mature reproductive tract; HT = hormone treatment using EC or EB.

For the variable, time from removal of the intravaginal device to estrous detection, the fixed model was:

$$Y_{ijk} = \mu + RTDi + HTj + e_{ijk}$$

where Y_{ijk} = time from removal to estrous detection associated to the k-th heifer; µ = mean of all observations; RTD = effect of the i-th RTD; HT = effect of the j-th hormonal treatment; and e_{ijk} = random residual effect.

All statistical analysis were carried out using the logistic and GLM procedures of SAS (SAS, 2009). The significance level was set at 5%.

RESULTS

The percentage of heifers in estrus and time from removal of the intravaginal device to estrus, was similar between mature and immature heifers (P>0.05; Table 2). Hormonal treatment had only effect on the percentage of heifers in estrus. Heifers treated with EB were 2.65 times more likely to present estrus compared to the group treated with EC (84.3% vs. 67.3%; P<0.05). However, the pregnancy rate after FTAI (74.8%) and total pregnancy (91.9%) were higher in the heifers with a mature reproductive tract (P<0.05). The pregnancy rate by NM was similar between mature and immature heifers (P>0.05; Table 2).

DISCUSSION

The percentage of heifers in estrus in the EB group was 17% higher than the EC group. This finding agree with Uslenghi et al. (2014), who reported a 20% increase in estrus in heifers treated with EB compared to heifers treated with EC. However, the results of the present study contrast those of Peralta-Torres et al. (2010b), who did not find differences between hormonal treatments (P>0.05). The greater proportion of heifers found for EB could be due its pharmacokinetic properties, because it is more rapidly absorbed and had a shorter time of action than EC hormone (Vynckier et al., 1990).

The overall mean time interval between intravaginal device removal and the beginning of estrus (42.6 h) was similar to the range (42-45 h) reported by Peralta-Torres et al. (2010b) but was smaller than the mean (49.3 h) reported by Colazo et al. (2003). Time from intravaginal device removal to estrus is important for establishing the best moment for FTAI (Ayres et al., 2008; Kasimanickam et al., 2015). Ovulation occurs 30 and 31 h after cows estrous manifestation when EC or EB is used (Peralta-Torres et al., 2010a). Therefore, when more prolonged is the beginning of estrous, after DIB withdrawal, the time for AI will be misaligned.

The pregnancy rate after FTAI was highest for heifers with a mature RTD, which is in agreement with Holm et al. (2009) results. These latter authors reported a higher pregnancy rate after FTAI in heifers with a mature reproductive tract (grades 5 and 4 with 80% and 70%, respectability) compared to heifers with an immature

Table 2: Means (±SE) by reproductive tract development and hormonal treatment for percentage of heifers in estrous, time from removal of intravaginal device to estrous, pregnancy rate after FTAI, pregnancy rate by NM and pregnancy rate after FTAI+NM in *Bos indicus* synchronized heifers

Items	Reproductive tract development		Hormonal treatment	
	Immature	Mature	Estradiol cypionate	Estradiol benzoate
Heifers (n)	101	99	98	102
Heifers in estrous (%)	71.3^{a}	80.8^{a}	67.4^{a}	84.3^{b}
Time from intravaginal device removal to estrous (h)	43.0^{a} ±0.75	42.3a ±0.71	43.0a ±0.78	42.2a ±0.67
Pregnancy after FTAI (%)	61.4^{a}	74.8^{b}	71.4^{a}	64.7^{a}
Pregnancy by NM (%)	18.8^{a}	17.1^{a}	13.3^{a}	22.6^{a}
Pregnancy after FTAI+NM (%)	80.2^{a}	91.9^{b}	84.7^{a}	87.3^{a}

FTAI: fixed time artificial insemination; NM: natural mating. ^{ab} Different literals between columns indicate statistical differences (P<0.05)

reproductive tract (grades 3, 2, and 1 with 53%, 40%, and 31%, respectability) (P<0.05). Kasimanickam et al. (2015) also reported an increased pregnancy rate in heifers with more mature RTD. However, Cutaia et al. (2006), Stevenson et al. (2008), and Butler et al. (2011) did not find an association (P>0.05) between stage of uterus development and pregnancy rate in heifers. The higher gestation rate of heifers with mature reproductive tract cpuld be related to a better development of the reproductive tract, as well as to the production of estradiol during the pre-ovulatory period (Claro et al., 2010; Souza et al., 2011). Estradiol causes proliferative changes in the uterine tissues, which are reflected in the growth and development of luminal and glandular epithelial cells, and the secretion of luminal proteins (Johnson et al., 1997).

No differences in pregnancy rate (P>0.05) were found between the EC and EB groups, which agrees with the results of Meneghetti et al. (2009) for Nellore cattle (50.8% and 51.9% pregnancy rate, respectively) and Uslenghi et al. (2016) for *Bos taurus* beef cows (54% and 49.2% pregnancy rate, respectively). However, Uslenghi et al. (2014) reports the effects of hormonal treatment on pregnancy rate, founding a higher rate after EB (61.5%) compared to EC treatment (48.9%). The results found in the present study indicate that the type of estrogen used does not influence the gestation rate; however, the advantage of EC is to reduce the management of heifers in programs using FTAI.

For the NM group, no differences were found in pregnancy rate (P>0.05), which agrees with Butler et al. (2011), who did not find any association between the stage of uterus development and pregnancy in naturally mated heifers. This result could address the ability of the treatment to induce of return to estrus in heifers that did not become pregnant at first insemination (Butler et al., 2011).

The total pregnancy rate (FTAI + NM) was greater for heifers with a mature reproductive tract (92%), which disagrees with Butler et al. (2011), who reported no differences in pregnancy rate for heifers with different RTD. The overall pregnancy rate found herein is similar to that reported by Gottschall et al. (2012), who reported 91.7% gestating females 100 d after beginning the study. Baruselli et al. (2004) and Salgado et al. (2009) found pregnancy rates of 79% at 90 d and 70.4% at 60 d, respectively.

Total pregnancy rate in the present study is also higher than the value (80.7%) obtained by Bó et al. (2007). In addition, Gutierrez et al. (2014) reported that heifers with more developed reproductive tracts had higher pregnancy rates than heifers with immature reproductive tracts.

CONCLUSIONS

Under the conditions of this study, *Bos indicus* heifers with mature reproductive tracts had higher pregnancy rate under fixed-time artificial insemination and higher total pregnancy than immature heifers.

Hormone treatment did not influence pregnancy rate; therefore, it is possible to use estradiol cypionate to reduce heifer handling.

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AUTHORS´ CONTRIBUTIONS

All the authors wrote, read and approved the manuscript. Jorge Alonso Peralta-Torres, Oswaldo Margarito Torres-Chablé, Nadia Florencia Ojeda-Robertos carried out the experiment, designed the research plan and organized the study. Carlos Luna-Palomera, Jesús Ricardo Aké-López and José Candelario Segura-Correa conducted the statistical analysis and interpretation of data.

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