OVULATION SYNCHRONIZATION PROTOCOLS AND FACTORS ASSOCIATED WITH PREGNANCY RATE OF ZEBU FEMALES †

[PROTOCOLOS DE SINCRONIZACIÓN DE LA OVULACIÓN Y FACTORES ASOCIADOS A LA TASA DE GESTACIÓN DE HEMBRAS CEBÚ]

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SUMMARY

Background. Fixed time artificial insemination is an important tool for the reproductive management of cattle. **Objective.** To determine the effect of two ovulation synchronization protocols and factors associated with the pregnancy rate of Zebu females using fixed time artificial insemination (FTAI) under tropical conditions. **Methodology**. Data of estrous and pregnancy from 129 multiparous Zebu cows and 257 heifers were used. All animals received an intravaginal device (new or used) CIDR® or DIB®, which remained *in situ* per 7 days. On the day of device removal, two treatments were established. I) Estradiol cypionate (EC; n = 192): 25 mg prostaglandin F2 alpha and 0.5 mg EC (estradiol cypionate); II) Estradiol benzoate (BE; n = 194): 25 mg $PGF_{2\alpha}$ and 24 hours later (day 8), 1 mg BE. Estrous was detected by 1-hour; three times a day, and females inseminated 54-56 hours after removal of device, using thawed semen. Pregnancy diagnosis was by transrectal palpation with real-time ultrasound, 60 days after FTAI. Data were analyzed using Chi-square tests. **Results.** The overall estrous rate was 73.32%. The ovulation synchronization protocol affected the presence of estrous, where EC had a higher rate (79.39%) compared to EB (67.19%). Pregnancy rate for the females inseminated with thawed semen was 54.40%. All factors affected the pregnancy rate ($p<0.05$). **Implications.** This study provides an alternative for increasing estrous and pregnancy rate of Zebu females, using EC synchronization protocol. It also shows that female type, device and BCS change influence pregnancy rate. **Conclusion**. The highest rate was observed in heifers showing estrous, synchronized with a new intravaginal device, using EC and gaining body condition score.

Keywords: body condition score; estradiol benzoate; estradiol cypionate; thawed semen; *Bos indicus*

RESUMEN

Antecedentes. La inseminación artificial a tiempo fijo es una herramienta importante en el manejo reproductivo del ganado. **Objetivo.** Determinar el efecto de dos protocolos de sincronización de la ovulación y factores asociados con la tasa de preñez de hembras Cebú, mediante inseminación artificial a tiempo fijo (IATF) bajo condiciones tropicales. **Metodología**. Se utilizaron los datos de estro y preñez de 129 vacas multíparas y 257 vaquillas Cebú. Todos los animales recibieron un dispositivo intravaginal (nuevo o usado) CIDR® o DIB®, que permaneció *in situ* durante 7 días. El día de la extracción del dispositivo, se establecieron dos tratamientos. I) Cipionato de estradiol (CE; n = 192): 25 mg de prostaglandina F2 alfa y 0.5 mg de CE; II) Benzoato de estradiol (BE; n = 194): 25 mg de $PGF_{2\alpha}$ y 24 horas después (día 8), 1 mg de BE. El estro se detectó por 1 hora, tres veces al día, y las hembras se inseminaron con semen descongelado 54-56 horas después de retirar el dispositivo. El diagnóstico de preñez fue por palpación transrectal con ultrasonido en tiempo real, 60 días después de la IATF. Los datos se analizaron mediante pruebas de Chi-cuadrado. **Resultados.** La tasa global de estro fue 73.32%. El protocolo de sincronización de la ovulación afectó la presencia de estros, donde CE tuvo una tasa más alta (79.39%) comparado con EB (67.19%). La tasa de preñez de las hembras inseminadas con semen descongelado fue 54.40%. Todos los factores afectaron la tasa de preñez ($p <0.05$). **Implicaciones.** Este estudio proporciona una alternativa para aumentar la tasa de estros y preñez en hembras Cebú, usando el protocolo de sincronización con CE. También muestra que el tipo de hembra, dispositivo y cambio de CC

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influyen en la tasa de preñez. **Conclusión.** La tasa más alta se observó en las vaquillas que mostraron estro, se sincronizaron con dispositivo nuevo, se le aplicó CE y que ganaron condición corporal.
Palabras clave: condición corporal; benzoato de estradiol; cipionato de estradiol; semen descongelado; *Bos indicus*

INTRODUCTION

Fixed time artificial insemination (FTAI) has been used as an important tool for reproductive management in cattle. Among the main advantages of FTAI are the calving of a group of females at the same time, and an increase of the weaning rate, in the best season of the year; in addition to, the rationalization of working time and improvement of the cost/benefit of the livestock activity (Sales *et al*., 2011; Uslenghi *et al*., 2016).

Today, the most commonly hormonal programs used to synchronize cows are based on progesterone and estradiol. Those programs have been successfully used in *Bos taurus* and *Bos indicu*s to synchronize ovulation, and to control the follicular and luteal phases of cows and heifers (Carvalho *et al*., 2008). However, pregnancy rates obtained under such conditions show a wide range of values (10 to 82%) especially under tropical conditions (Bó and Baruselli, 2014). Several factors influence reproductive success following FTAI protocols in cattle (Meneghetti *et al*., 2009; Sá-Filho *et al*., 2009; Galvão and Santos, 2010). Some authors mention the use of new or reused intravaginal devices (Sales *et al*., 2015), hormonal treatments (Ambrose *et al*., 2005; Sales *et al*., 2012; Torres-Júnior *et al*., 2014), follicle size or diameter (Sá Filho *et al*., 2010), and poor body condition score (BCS) at estrous synchronization (Nishimura *et al*., 2018). Therefore, further studies about FTAI to improve pregnancy rate and factors associated under tropical systems of production are needed. The main objective of this study was to determine the effect of ovulation synchronization protocols and factors associated with pregnancy rate of *Bos indicus* females, inseminated at a fixed time, under humid-tropical conditions of México.

MATERIALS AND METHODS

Description of the study area

The experiment was carried out in a production unit located at the central region of the Villahermosa, Tabasco, Mexico, located 18° 20'N and 93º 15'W, and at 20 m above sea level. The climate of the region is warm-humid with rain in summer, with an average annual temperature of 28.7 °C and an average annual rainfall of 1,940.6 mm (INEGI, 2014).

Type of study and animals

A retrospective study (2012 and 2013) was carried out, collecting the estrous and pregnancy information of 386 *Bos indicus* females (commercial Brahman). Of them, 129 were weaned multiparous cows (451 ± 30.5 Kg and 4-5 calvings) with 180 and 240 days postpartum; and 257 were heifers (355 ± 28.6 Kg) between 24 and 30 months of age. The females had an average body condition score (BCS) of 4.74 $\pm$ 0.84, where 1 = emaciated and 9 = obese (Ayala *et al*., 1995). The animals were kept in paddocks of African Star grass (*Cynodon nlemfuensis*), 24 hours a day, and received 1 kg/ animal/ day of a commercial feed, fresh based, with 14% crude protein.

Treatments

At the start of the study (day 0), all females were applied a new or used (single use) intravaginal device (CIDR® Pfizer laboratory, Mexico) or DIB® (Syntex laboratory, Argentina), which contained 1.9 or 1 g natural progesterone, respectively, plus 2 mg of estradiol benzoate applied intramuscularly (Syntex laboratory, Mexico). The CIDR or DIB remained *in situ* for 7 days. On the day of removal of the device (day 7), two treatment groups were established (Figure 1):

I) Estradiol cypionate (EC; n = 192): 25 mg prostaglandin F2 alpha (Dinoprost tromethamine; Lutalyse®, Pharmacia & Upjohn, Mexico) and 0.5 mg of EC were applied (Syntex, laboratory, Argentina), both intramuscular via.

II) Estradiol benzoate (EB; n = 194): 25 mg $PGF_{2\alpha}$ was applied and 24 hours later (day 8) 1 mg of EB, both intramuscular via.

Estrous detection, FTAI and pregnancy diagnosis

Estrous was detected by visual observation, three times a day (06:00, 12:00 and 18:00 h), per 1 hour, starting 24 hours after the removal of the intravaginal device, and ending at the time of FTAI (Peralta-Torres *et al*., 2019). Females were inseminated with frozen-thawed semen by the same technician, 54-56 hours after removal of the device. Semen straws were thawed at 35ºC for 30 s prior to loading the insemination tool. The entire semen dose (0.25 mL straws) was placed in the body of the uterus. All batches of semen used (red Angus and Brahman) were analyzed prior to the study

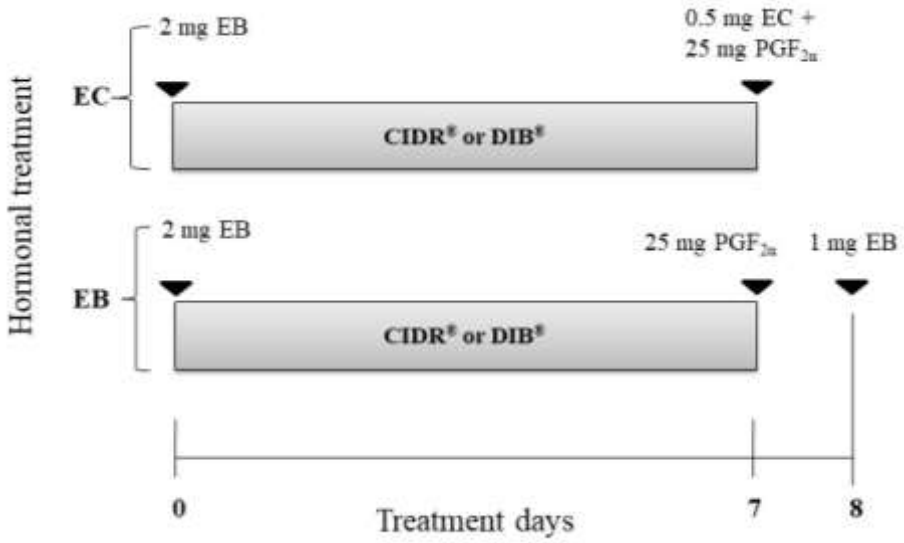

Figure 1. Synchronization program in *Bos indicus* females. EC (estradiol cypionate), EB (estradiol benzoate), $PGF_{2\alpha}$ (Dinoprost tromethamine), CIDR® (Controlled Internal Drug release), DIB® (intravaginal device bovine).

and have post-thaw concentration of 15×10^6 sperm/mL. The diagnosis of pregnancy was made by transrectal palpation with a real-time ultrasound (Emperor®-830 Vet, China), 60 days after FTAI.

Evaluation of BCS

The BCS of each female was evaluated according to Ayala *et al*. (1995), the day of insertion of the device (day 0) and during the diagnosis of pregnancy (day 60 post-FTAI). BCS change was calculated as the difference between BCS the day of insertion of the device and BCS at the pregnancy diagnosis. Three categories of BCS were established: female lost, maintained or gained BCS.

Statistical analysis

The presence of estrous (1 = estrous, 0 = no estrous) and pregnancy diagnosis (1 = pregnant; 0 = empty) were analyzed by factor, using Chi-square procedures (SAS, 2012). Pregnancy rates are provided and a significance level of 5% was used. No multifactorial analysis with interaction (logistic regression) were carried out because of small number of observation per one-way combination of the levels of the factors.

RESULTS

The overall estrous rate was 73.32%. The single risk factor that influenced the presence of estrous was treatment, where estradiol cypionate had a higher rate (79.38%) compared to estradiol benzoate (67.19%).

Table 1. Pregnancy rate by factor for *Bos indicus* synchronized females under tropical conditions.

Factor	n	Pregnancy (%)	Chi^2 p-value
Estrus			<0.0001
Yes	176	55.17	
No	75	23.51	
Type of female			0.0010
Heifer	257	60.31	
Cow	129	42.64	
Device			<0.0001
New	287	60.63	
Used	99	36.36	
Treatment			0.0183
EC	192	60.42	
EB	194	48.45	
BCS change			<0.0001
Loss BCS	71	35.21	
Kept BCS	164	48.20	
Gained BCS	151	70.20	

BCS: Body condition score; EC: estradiol cypionate; EB: estradiol benzoate.

The overall pregnancy rate of the females inseminated with thawed semen was 54.40%. The relative frequencies and Chi-square results of the studied factors are given in Table 1. All factors showed significant effect on pregnancy rate ($p<0.05$). Heifers, females showing estrous, those using a new intravaginal device and females that increase BCS had higher pregnancy rate.

DISCUSSION

Expression of estrous during an estradiol/progesterone-based FTAI protocol has been found to increase fertility and diminish pregnancy loss (Souza *et al*., 2007; Pereira *et al*., 2016). In the present study, the highest proportion of females in estrous was for the group with EC; this due to the different kinetics of the hormone (Vynckier *et al*., 1990), which exerts action in a shorter time compared to EB. Although the synchronization protocols for FTAI do not depend on estrous detection, it is of great importance that a large percentage of females presented estrous, ≤ 52 hours after removal of the intravaginal device, in order to ovulation occurs, cows get fertilized and pregnancy happens (Peralta-Torres *et al*., 2019).

In synchronization protocols using new devices, a higher percentage of females in estrus are commonly observed (80%) in comparison with 20% lower percentages in used device (Uslenghi *et al*., 2014). However, there was the possibility that a low percentage of females were not observed in estrus because estrous in *Bos indicus* cattle has a short life and nocturnal manifestations (Galina and Arthur, 1990). The females observed in estrus in an IATF program usually increase the success of pregnancy rate.

The proportion of females who showed estrous and got pregnant (Table 1), agree with Sá-Filho *et al*. (2010) results. They observed a higher pregnancy rate in females that showed estrous. Displaying estrous at the end of an estradiol/progesterone-based protocol could be related to a reduction of progesterone concentrations near AI, and to an increase of estradiol during pro-estrous. This due to estradiol esters and endogenous estradiol from the ovulatory follicle (Pereira *et al*., 2014). The prolonged exposure to estradiol during pro-estrous, may increase plasma progesterone concentration, mRNA expression of IFNt-related genes in 15-day old conceptuses, and provide a better environment for pregnancy maintenance (Binelli *et al*., 2014; Cooke *et al*., 2019).

Heifers showed higher pregnancy rate (60.31%) than cows. However, most studies in the literature indicates that the highest value occurs in cows (Sá-Filho *et al*., 2009; Nogueira *et al*., 2014; Simões *et al*., 2018) or no differences are found (Meneghetti *et al*., 2009; Sá-Filho *et al*.,2010). However, the body condition of heifers at first calving plays an important role on pregnancy rate, as is discussed later.

The pregnancy rate in this study was similar to that reported by other authors (Colazo *et al*., 2004; Pereira *et al*., 2018), where the pregnancy rate was higher when new intravaginal devices were used. Thus, females subject to estrous synchronization protocols with low concentration of circulating progesterone (previously used implant), could have influenced the size of the follicle at the time of removal of the device or ovulation, resulting in a reduction in the pregnancy rate. However, although this study was carried out in a single production unit, it is important to mention that each group of females were of synchronized considering the season of year (dry, rainy and north), feeding, postpartum days, which are some factors to consider under the humid tropical conditions.

The overall pregnancy rate here found is within the range of values reported by Bó and Baruselli (2014), who indicate a range between 51 and 60%, using protocols with progesterone and estradiol. Females synchronized with EC showed the highest pregnancy rate, which differ from the results of other authors (Meneghetti *et al*., 2009; Sales *et al*., 2012; Torres-Júnior *et al*., 2014; Uslenghi *et al*., 2014), who indicate that there are no differences between both hormones. The EC is an ester of estradiol with low water solubility that delays its release from the site of injection (Colazo *et al*., 2003; Sá-Filho *et al*., 2011), and which could have synchronized the increase in LH and ovulation. Therefore, EC is an interesting alternative to replace EB in FTAI protocols to reduce animal handling without reduced fertility. Other option to increase in 20% the pregnancy rate with those protocols of synchronization is the used of cool semen (25×10^6 spermatozoa) in substitution of frozen-thawed semen (Borges-Silva *et al*., 2016).

The BCS of the females at the beginning of the synchronization protocol (day 0) is very important. Several studies indicate that low BCS reduces fertility (Sá-Filho *et al*., 2010, Sá-Filho *et al*., 2011, Sales *et al*., 2012, Pereira *et al*., 2018). However, as shown here, changes in BCS after the hormonal protocol and FTAI are relevant. In this sense, Carvalho (2017) reports that, animals that lost BCS after calving and before diagnosis of pregnancy had lower pregnancy rate when compared to animals that maintained or gained BCS. Few studies evaluate the changes of BCS from the beginning of hormonal treatment until the diagnosis of pregnancy (Peralta-Torres *et al*., 2019). Therefore, females should be kept in good nutritional conditions, to get a pregnancy rate of 70% or more, as was observed here.

Some factors evaluated here influenced the gestation rate, whereas others such as the inseminator, the concentration of the semen used and the production unit were controlled. Therefore, the results presented here depend mainly on the factors studied.

CONCLUSION

The highest pregnancy rate was observed in heifers, females showing estrous, those with a new intravaginal device, given EC and gaining BCS. Therefore, these factors must be considered when implementing FTAI protocols under humid tropical weather, where the environmental conditions sometimes are adverse and could negatively influence the pregnancy rate.

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Conflict of interest. The authors of this paper declare no conflict of interest.

Compliance with ethical standards. All study procedures were conducted according to the Mexican Official Standard NOM-062-ZOO-1999 for the production, care, and use of experimental animals.

Data availability. Data are available with Jorge Peralta-Torres, japt@hotmail.com upon request.

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